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In re the Application of: Noriaki KATO, Hiroshi NAGANO, Kaori TANIKO and
Takahito JOMORI

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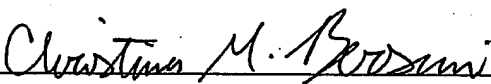
Conf. No.: 4731

For: PROPHYLACTIC OR THERAPEUTIC AGENT FOR DIABETIC
MACULOPATHY

Commissioner for Patents
P.O. Box 1450
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**CERTIFICATION OF EFS
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I hereby certify that this paper is being transmitted via EFS to the Patent
and Trademark Office on July 16, 2008.


Christina M. Bersani

DECLARATION UNDER 37 CFR §1.132

Sir:

I, Noriaki Kato, a citizen of Japan, hereby declare and state:

1. I graduated from Kanazawa University Faculty of Pharmaceutical Sciences in
Japan, in 1984.

2. I have been employed by Sanwa Kagaku Kenkyusho Co., Ltd., since 1984 and
I have had a total of 24 years of work researching drugs on diabetic cataract, diabetic
neuropathy and diabetic retinopathy.

3. I am a member of 1) The Japan Diabetes Society, 2) The Japan Society of
Diabetic Complications, 3) Japanese Ophthalmological Society and 4) Japan Society of
Experimental Diabetes and Obesity.

4. I am one of the inventors of the above-identified patent application and I am familiar with the references applied in the Office Action mailed March 18, 2008.

5. My publications include the following works in this field:

“Continuous inhibition of excessive polyol pathway flux in peripheral nerves by aldose reductase inhibitor fidarestat leads to improvement of diabetic neuropathy,” J. Diabetes Complications, 1999 May-Jun, 13(3):141-50.

“Effects of 15-month aldose reductase inhibition with fidarestat on the experimental diabetic neuropathy in rats, Diabetes Research and Clinical Practice,” Volume 50, Issue 2, 1 October 2000, Pages 77-85.

“Serial changes of sensory nerve conduction velocity and minimal F-wave latency in streptozotocin-induced diabetic rats,” Neuroscience Letters, Volume 244, Issue 3, 20 March 1998, Pages 169-172.

“Effect of long-term treatment with a new aldose reductase inhibitor, (2S,4S)-6-fluoro-2',5'-dioxospiro-[chroman-4,4'-imidazolidine]-2-carbox amide (SNK-860), on peripheral neuropathy in streptozotocin-induced diabetic rats,” J. Diabetes Complications, 1994 Jan-Mar, 8(1):27-32.

“Long-term treatment with fidarestat suppresses the development of diabetic retinopathy in STZ-induced diabetic rats,” Journal of Diabetes and its Complications, Volume 17, Issue 6, November-December 2003, Pages 374-379.

“Sorbitol dehydrogenase overexpression potentiates glucose toxicity to cultured retinal pericytes,” Biochemical and Biophysical Research Communications, Volume 299, Issue 2, 29 November 2002, Pages 183-188.

“Aldose Reductase Inhibitors,” Journal of Enzyme Inhibition, Volume 16, Issue 6 2001, pages 465 - 473.

“Effect of the aldose reductase inhibitor fidarestat on experimental diabetic neuropathy in the rat,” Diabetologia, Volume 49, Issue 12, December 2006, pages 3085-3093.

6. The following experiments were conducted by me or under my direct supervision.

The experiment is composed of three tests: (A) pharmacological preliminary test 1 using crab-eating monkeys; (B) pharmacological preliminary test 2 using crab-eating monkeys; and (C) pharmacological test using crab-eating monkeys of experimental diabetes.

A. Pharmacological preliminary test 1 using crab-eating monkeys

-Epalrestat 400 mg/kg/day short-term repeated administration test-

1. Purpose

The purpose of this test is to set a maximum effective dose of epalrestat used in macular edema evaluation study with streptozotocin (STZ) induced diabetic crab-eating monkey. The toxic dose level of epalrestat in the dog 3-month sub-acute toxicity test is estimated to be 500 mg/kg/day. A decreasing trend of the body weight is perceived at a 22nd day of administration by this dose¹⁾. Based on the foregoing report, repeated administration of 400 mg/kg/day for 5 days was determined.

2. Experimental material and method

2.1. Test article

Epalrestat (Lot No. EPA-06510, Sanyo Chemical Laboratory Co., Ltd.) was employed as a test article.

2.2. Test method

2.2.1. Grouping

Four normal male crab-eating monkeys (*Macaca fascicularis*) from Nafovanny, Vietnam, ranging from 2 and 3 years old, were divided into two groups each of which was composed of two.

1) Control group (two monkeys)

2) Epalrestat 400 mg/kg/day administrated group (two monkeys)

2.2.2. Medication administration and blood drawing

A catheter was inserted from the buccal cavity through the esophagus into the stomach so that an epalrestat suspension (prepared by 5% gum arabic solution) was administered. Administration was carried out three times a day (in the morning, daytime and at night) and repeated for five days. A 5% gum arabic solution was orally administered to the control group in a ratio of 5 ml/kg. The general condition and body weight were monitored every day in the administration period. Furthermore, blood was drawn from the saphena immediately before administration and five days after the administration using a disposable syringe barrel and needle without use of an anesthetic. Drawn blood was put into a Venoject II (Terumo Corporation) and left for 40 to 60 minutes at a room temperature. A biochemical examination using obtained blood serum was entrusted to the Corporation for Production and Research of Laboratory Primates.

3. Experimental results

3.1. General condition

No significant changes in the condition were found in each individual of the control group during the test. In the test article administered group, excretion of test article color-like urine was found from a first to fifth administration days regarding each individual. One of the monkeys had about 2 mL vomit of test article-like substance five days after administration, and another monkey had about 3 mL vomit of test article-like substance four and five days after administration.

3.2. Body Weight

TABLE 1 shows body weight changes. No significant changes in the body weight were found in each individual of the control group during the test. In the test article administered group, one of the monkeys had weight loss of 0.2 kg five days after administration, and another monkey had weight loss of 0.3 kg five days after administration. The monkey which had the weight loss of 0.2 kg during five days died five days after cessation of the test article.

3.3. Biochemical examination of blood serum

TABLE 2 shows results of the examination. No changes were found in the control group before and after the administration. In the test article administered group, changes were found in each of BUN, GPT, LDH, CRE and T-BIL.

4. Summary

400 mg/kg/day epalrestat was considered to be a toxic dose based on aggravation of general condition, weight loss and variations in hepatic and kidney damage markers.

5. Reference

Katsumasa ISHIMURA, Hitoshi OGAWA, Koichi ISOWA, Hidetoshi KASHIMA and Shinji WATANABE, et al., "Three-Month Subacute Toxicity Test and One-Month Recovery Test in Dogs by Oral Administration of ONO-2235," Modern Medical Care (Gendai-Iryou), 1986; 18 (Supplement III); p. 162-231 (attached as Document 5 hereto).

TABLE I
CHANGE IN BODY WEIGHT

Group	Dose (mg/kg)	Animal	First day	Second day	Third day	Fourth day	Fifth day
Control	0	(1)	2.4	2.3	2.3	2.3	2.3
		(2)	2.4	2.4	2.4	2.4	2.4
Epalrestat	400	(1)	2.5	2.5	2.5	2.5	2.3
		(2)	2.5	2.4	2.4	2.3	2.2

TABLE 2

BIOCHEMICAL EXAMINATION OF BLOOD SERUM

Group	Dose (mg/kg)	Animal	BUN (mg/dL)	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	LDH (IU/L)
Control	0	(1) B	18.8	26	29	1717	244
		(2) B	23.8	21	16	2143	234
Epalrestat 400	400	(1) B	20.0	29	46	1934	270
		(2) B	22.1	31	38	2546	233
Control	0	(1) A	20.8	27	35	2009	289
		(2) A	27.8	25	21	2322	300
Epalrestat 400	400	(1) A	39.1	43	104	2138	778
		(2) A	26.0	38	48	1914	287

where "B" designates the value before administration and "A" designates the value after administration for five days; and where BUN designates blood urea nitrogen, GOT glutamic-oxaloacetic transaminase, GPT glutamic-pyruvic transaminase, ALP alkaline phosphatase, LDH lactate dehydrogenase, CRE Creatinine and T-BIL total bilirubin.

TABLE 2 -Continued-

BIOCHEMICAL EXAMINATION OF BLOOD SERUM

Group	Dose (mg/kg)	Animal	CRE (mg/dL)	T-BIL (mg/dL)
Control	0	(1) B	0.5	0.2
		(2) B	0.6	0.4
Epalrestat 400	400	(1) B	0.4	0.2
		(2) B	0.5	0.2
Control	0	(1) A	0.5	0.2
		(2) A	0.5	0.3
Epalrestat 400	400	(1) A	1.5	0.4
		(2) A	1.1	0.7

B. Pharmacological preliminary test 2 using crab-eating monkeys

-Epalrestat 150 mg/kg/day short-term repeated administration test-

1. Purpose

The purpose of this test is to set a maximum effective dose of epalrestat used in macular edema evaluation study with streptozotocin (STZ) induced diabetic crab-eating monkey. Since a toxic action was seen in the epalrestat 400 mg/kg/day short-term repeated administration test, inspection was carried out with the dose being reduced to 150 mg/kg/day.

2. Experimental material and method

2.1. Test article

Epalrestat (Lot No. EPA-06510, Sanyo Chemical Laboratory Co., Ltd.) was employed as a test article.

2.2. Test method

2.2.1. Grouping

Three normal male crab-eating monkeys (*Macaca fascicularis*) from Nafovanny, Vietnam, ranging from 2 and 3 years old, were used. Only one medication administration group was set in order that toxic action may be detected before and after administration.

1)Epalrestat 150 mg/kg/day administrated group (three monkeys)

2.2.2. Medication administration and blood drawing

A catheter was inserted from the buccal cavity through the esophagus into the stomach so that an epalrestat suspension (prepared by 5% gum arabic solution) was administered. Administration was carried out three times a day (in the morning, daytime and at night) and repeated for six days. The general condition and body weight were monitored every day in the administration period. Furthermore, blood was drawn from the saphena or femoral vein immediately before administration and six days after the administration using a disposable syringe barrel and needle without use of an anesthetic. Drawn blood was put into a Venoject II (Terumo Corporation) and left for 40 to 60 minutes at a room temperature. A biochemical

examination using obtained blood serum was entrusted to the Corporation for Production and Research of Laboratory Primates.

3. Experimental results

3.1. General condition

In all the individuals, excretion of test article color-like urine was found from a first to sixth administration days.

3.2. Body Weight

TABLE 3 shows body weight changes. All the individuals had weight loss of 0.1 to 0.2 kg five or six days after administration.

3.3. Biochemical examination of blood serum

TABLE 4 shows results of the examination. By treatment with test article, changes were found in each of BUN, GPT, LDH and CRE.

4. Summary

Mild but temporal weight loss was seen and variations in hepatic and kidney damage markers were also seen, and accordingly, 150 mg/kg/day epalrestat was also considered to be a toxic dose.

TABLE 3

CHANGE IN BODY WEIGHT

Group	Dose (mg/kg)	Animal	First day	Second day	Third day	Fourth day	Fifth day	Sixth day
Epalrestat	150	(1)	2.3	2.3	2.2	2.2	2.1	2.1
		(2)	2.3	2.4	2.3	2.2	2.1	2.1
		(3)	2.4	2.4	2.4	2.4	2.3	2.3

TABLE 4

BIOCHMICAL EXAMINATION OF BLOOD SERUM

Group	Dose (mg/kg)	Animal	BUN (mg/dL)	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	LDH (IU/L)
Epalrestat 150		(1) B	19.4	34	17	2782	266
		(2) B	19.4	36	30	1812	248
		(3) B	16.5	44	19	2186	366
		(1) A	46.6	57	139	2139	783
		(2) A	46.9	35	58	1421	575
		(3) A	15.7	27	17	2358	342

where "B" designates the value before administration and "A" designates the value after administration for six days; and where BUN designates blood urea nitrogen, GOT glutamic-oxaloacetic transaminase, GPT glutamic-pyruvic transaminase, ALP alkaline phosphatase, LDH lactate dehydrogenase, CRE Creatinine and T-BIL total bilirubin.

TABLE 4 -Continued-

BIOCHMICAL EXAMINATION OF BLOOD SERUM

Group	Dose (mg/kg)	Animal	CRE (mg/dL)	T-BIL (mg/dL)
Epalrestat 150		(1) B	0.5	0.2
		(2) B	0.5	0.2
		(3) B	0.5	0.2
		(1) A	4.9	0.2
		(2) A	2.9	0.1
		(3) A	0.9	0.2

C. Pharmacological study using experimental diabetic crab-eating monkeys

-Action of SNK-860 and epalrestat against macular edema due to ischemic reperfusion-

The effectiveness of aldose reductase inhibitor on macular edema due to ischemic reperfusion was compared regarding SNK-860 and epalrestat using streptozotocin (STZ) induced diabetic crab-eating monkeys (*Macaca fascicularis*).

2. Experimental material and method

2.1. Diabetes inducing substance and insulin

Diabetes mellitus was induced in monkeys by intravenously injecting streptozotocin (STZ: Lot No. 046K1206, Sigma) into their foreleg vein at the dose of 80 mg/kg. Insulin (NOVOLIN (registered trademark), NovoNordisk A/S) was administered subcutaneously twice a day (before feeding in the morning and in the evening). An applied dose of insulin was determined to be a slight amount necessary for subsistence (0.01 to 0.05 mL/body).

2.2. Test article

SNK-860 (Lot No. 901T-4) was employed as a test article. It is reported that SNK-860 suppresses 50% sorbitol accumulation at the dose of 2 mg/kg and 100% sorbitol accumulation at the dose of 16 mg/kg. Based on the report, the dose sufficiently suppressing sorbitol accumulation was set at 4 mg/kg/day and 8 mg/kg/day. Administration was carried out once a day (in the morning) for 14 days before increase in the intraocular pressure and for 7 days after increase in the intraocular pressure.

2.3. Control article

Epalrestat (Lot No. EPA-06510, Sanyo Chemical Laboratory Co., Ltd.) was employed as a control article. The dose was set at 25 mg/kg/day and 50 mg/kg/day based on the experiment results of preliminary test 1 and 2, and the report that 73% sorbitol accumulation was suppressed in the retina of a diabetic model at the dose of 50 mg/kg/day. Administration was carried out three times a day (in the morning, daytime and at night) for 14 days before increase in the intraocular pressure and for 7 days after increase in the intraocular pressure.

2.4. Test method

2.4.1. Grouping

STZ was administered to 20 male crab-eating monkeys (*Macaca fascicularis*) from Nafovanny, Vietnam, ranging from 2 and 3 years old, to induce diabetes mellitus. Surviving 16 monkeys were divided into four groups. The grouping was carried out three weeks after administration of STZ in view of glycated hemoglobin levels, body weight and results of general condition before administration of medications (test article or control article). Groups were as follows:

- 1) Diabetic control group (4 monkeys);
- 2) Diabetic group of given 4 mg/kg SNK-860 (3 monkeys);
- 3) Diabetic group of given 8 mg/kg SNK-860 (3 monkeys);
- 4) Diabetic group of given 25 mg/kg epalrestat (3 monkeys); and
- 5) Diabetic group of given 50 mg/kg epalrestat (3 monkeys).

2.4.2. Preparation of retinal ischemia by increase of the intraocular pressure

Intraocular pressure was applied to both eyes of each monkey at a 14th day of administration by the following method:

A drip infusion set (Terufusion Drip Infusion Set manufactured by Terumo) was connected to a bottle containing an intraocular perfusion solution (Opeguard MA manufactured by Senju Pharmaceutical Co., Ltd.), and an extension tube to which a three-way stopcock had been attached was connected thereto. A needle was fitted to the end of the tube. The bottle was fixed to a stand so that a liquid level of the intraocular perfusion solution is 184 cm high relative to monkey's eyes. A mydriatic (Mydrin P manufactured by Santen Pharmaceutical Co., Ltd.) was dropped onto monkey's eye with sufficient mydriatic action. Thereafter, Ketalar (Sankyo Lifetech Co., Ltd.) was administered into muscle for anesthesia. Subsequently, a local anesthetic agent (Benoxyl eye drop 0.4%, Santen Pharmaceutical Co., Ltd.) was dropped and a lid retractor was then attached to prevent blinking. Ketalar was timely added. Thereafter, a needle was stuck into an anterior chamber of the monkey's eye and the three-way stopcock was operated so that pressure is applied in the eye, whereby a retina ischemic condition was

prepared. An ischemic time by application of intraocular pressure was set to 60 minutes. After application of the intraocular pressure, the needle was removed to relieve the intraocular pressure to allow reperfusion, and an antibacterial eye drop (tarivit eye ointment manufactured by Santen Pharmaceutical Co., Ltd.) was applied onto the eye. The thickness of macula was measured using an OCT scanner (Stratus OCT, Carl Zeiss Meditec AG) two days after application of intraocular pressure (16 days after medication administration) and four days after application of intraocular pressure (18 days after medication administration). Evaluation was carried out without consideration of eyeballs to which insufficient intraocular pressure was applied and in which macula was not observed by the OCT scanner.

2.4.3. Measurement of macula thickness

Macula cross-sectional imaging of the monkey under anesthesia was photographed using an OCT scanner on a fourteenth day from medication administration (immediately before application of intraocular pressure), a sixteenth day (two days after application of intraocular pressure) and eighteenth day (four days after application of intraocular pressure). Evaluation was carried out with respect to the minimum thickness of macula center.

2.4.4. Dissection

The monkeys were normally raised after application of intraocular pressure. Measurement of body weight and blood drawing were carried out 21 days after medication administration (seven days after application of intraocular pressure) and thereafter, dissection was also carried out. Glycated hemoglobins were measured using drawn blood.

3. Test results

3.1. Effect on macular edema

TABLE 5 shows the results. A macular edema of diabetic monkey (an increase in the minimum thickness of macula center) was produced by application of intraocular pressure. The macular edema was seen during four days after application of intraocular pressure. Significant edema suppressing effect was admitted in the diabetic monkeys to which 4 or 8 mg/kg/day SNK-860 was administered. On the other hand, no significant edema suppressing

effect was admitted in the diabetic monkeys to which 25 or 50 mg/kg/day epalrestat was administered.

3.2. Effect on body weight and glycated hemoglobin

TABLE 6 shows the results. No difference was seen among groups regarding body weight and glycated hemoglobin. Furthermore, the weight and hemoglobin were increased in all groups as compared with the time of grouping.

4. Summary

1) SNK-860 exhibited stronger suppression of macular edema in diabetes mellitus than epalrestat.

2) No effect was seen even when the dose of epalrestat was increased and accordingly, the effectiveness thereof against macular edema in diabetes mellitus was unclear. Additionally, the set dose (25 and 50 mg/kg/day) was considered to have no controversial toxic effect.

3) The level of hemoglobin was rendered larger at the time of final administration than at the time of grouping, regarding all groups. Accordingly, the medicinal effect was considered to be brought by each medication but not by a blood glucose improving effect of insulin.

5. Reference

I.G. Obrosova, A.G. Minchenko, R. Vasupuram, L. White, O.I. Abatan, A.K. Kumagai, R.N. Frank and M.J. Stevens, "Aldose reductase Inhibitor Fidarestat Prevents Retinal Oxidative Stress and Vascular Endothelial Growth Factor Overexpression in Streptozotocin-Diabetic Rats," *Diabetes* 2003; 52: 864-871 (listed herein as Document 6).

N. Hotta, H. Kakuta, H. Fukasawa, M. Kimura, N. Koh, M. Iida, H. Terashima, T. Morimura and N. Sakamoto, "Effects of a Fructose-Rich Diet and the Aldose Reductase Inhibitor, ONO-2235, on the Development of Diabetic Neuropathy in Streptozotocin-Treated Rats," *Diabetologia* 1985; 28; 176-180 (attached hereto as Document 7).

TABLE 5

MINIMUM THICKNESS OF MACULAR CENTER AFTER APPLICATION OF
INTRAOCULAR PRESSURE

Group	Dose (mg/kg)	Number of eyes	Before application of intraocular pressure
Diabetic control	-	7	135±3
SNK-860	4	5	128±5
	8	6	131±5
Epalrestat	25	4	130±6
	50	6	128±4

TABLE 5 -Continued-

MINIMUM THICKNESS OF MACULAR CENTER AFTER APPLICATION OF
INTRAOCULAR PRESSURE

Group	Application of intraocular pressure	
	Two days after	Four days after
Diabetic control	182±15 #	152±6 #
SNK-860	139±10 *	135±8
	140±7 *	138±5
Epalrestat	164±22	152±9
	189±31	161±15

where average±standard error (μm), #p<0.05 versus before application of intraocular pressure, and *p<0.05 versus diabetic control.

TABLE 6

BODY WEIGHT AND GLYCATED HEMOGLOBIN

Group	Dose (mg/kg)	Number of monkeys	Body Weight (kg)	
			Grouping	Final
Diabetic control	-	4	2.2±0.1	2.4±0.2
SNK-860	4	3	2.3±0.4	2.4±0.4
	8	3	2.1±0.0	2.2±0.0
Epalrestat	25	3	2.2±0.2	2.3±0.1
	50	3	2.2±0.1	2.2±0.1

TABLE 6 -Continued-

BODY WEIGHT AND GLYCATED HEMOGLOBIN

Group	Glycated hemoglobin (%)	
	Grouping	Final
Diabetic control	7.7±0.8	8.2±0.7
SNK-860	7.5±0.6	8.8±0.7
	8.0±0.2	9.2±0.2
Epalrestat	8.5±0.1	9.1±0.5
	7.9±1.8	8.8±1.8

where grouping designates "at the time of grouping" and final designates "at the time of final medication administration" and average±standard error

7. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and/or imprisonment under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Date: 16 July 2008 Noriaki Kato
Noriaki KATO

Attachments:

- Document 1: M.A. Speicher et al., "Pharmacologic Therapy for Diabetic Retinopathy," Expert Opinion on Emerging Drugs 2003; 8; 239-250 (no copy attached, cited as reference AH on the 1449 form included with the IDS filed July 26, 2006).
- Document 2: M. Akita et al., "Effects of an Aldose Reductase Inhibitor, SNK-860, on the Histopathological Changes of Retinal Tissues in a Streptozotocin-Induced Diabetic Rat Model," Acta Med Okayama 1993; 47: 299-304 (no copy attached, cited as reference AI on the 1449 form included with the IDS filed July 26, 2006).
- Document 3: JP-A-H07-242547 (no copy attached, cited as reference AC on the 1449 form included with the IDS filed July 26, 2006).
- Document 4: Hidetoshi Yamashita and Ryo Kawasaki ed., "Diabetic Retinopathy: Best Advice By Medical Specialist," Tokyo, Shindann-to-Chiryousha, 2003, pages 47-59.
- Document 5: Katsumasa ISHIMURA, Hitoshi OGAWA, Koichi ISOWA, Hidetoshi KASHIMA and Shinji WATANABE, et al., "Three-Month Subacute Toxicity Test and One-Month Recovery Test in Dogs by Oral Administration of ONO-2235," Modern Medical Care (Gendai-Iryou), 1986; 18 (Supplement III); p. 162-231.
- Document 6: I.G. Obrosova, A.G. Minchenko, R. Vasupuram, L. White, O.I. Abatan, A.K. Kumagai, R.N. Frank and M.J. Stevens, "Aldose Reductase Inhibitor Fidarestat Prevents Retinal Oxidative Stress and Vascular Endothelial Growth Factor Overexpression in Streptozotocin-Diabetic Rats," Diabetes 2003; 52: 864-871 (no copy attached, cited as reference AJ on the 1449 form included with the IDS filed July 26, 2006).
- Document 7: N. Hotta, H. Kakuta, H. Fukasawa, M. Kimura, N. Koh, M. Iida, H. Terashima, T. Morimura and N. Sakamoto, "Effects of a Fructose-Rich Diet and the Aldose Reductase Inhibitor, ONO-2235, on the Development of Diabetic Neuropathy in Streptozotocin-Treated Rats," Diabetologia 1985; 28; 176-180.

糖尿病網膜症

専門医によるベストアドバイス

編著

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5 糖尿病網膜症の重症度分類

A はじめに——糖尿病網膜症の進展様式

糖尿病網膜症（以後、網膜症）は高血糖に伴うさまざまな代謝異常によって引き起こされる網膜血管の障害を中心にみられる網膜障害である。早期には網膜出血や毛細血管瘤により発症する。さらに毛細血管からの漏出は硬性白斑として見られ、毛細血管床の閉塞は軟性白斑や静脈の異常などの網膜の虚血性変化をもたらす。虚血の程度が強くなると網膜新生血管が発生し硝子体出血などの原因となる。網膜新生血管の周囲には線維血管増殖膜が形成され牽引性網膜剥離をひき起こし重篤な視力障害を来す。また、虹彩や隅角に新生血管が発生すると血管新生緑内障を発症しさらに治療が困難となる。また網膜症自体はそれほど重症ではなくとも網膜黄斑部に黄斑浮腫等の黄斑症を伴うこともあり、その場合、失明には至らないまでも視力障害が引き起こされる。

B 糖尿病網膜症の分類

1. Davis 分類（表 1）

1965 年、Davis は網膜症と硝子体の関係を論じた論文の中で網膜症の分類を報告し¹⁾、それが現在まで Davis 分類として知られている。この分類では網膜症を、非増殖網膜症（網膜新生血管を認めない）と増殖網膜症（網膜新生血管を認める）に大別した。非増殖網膜症は軽症、中等症、重症に分けられている。特に軟性白斑や網膜内細小血管異常（intraretinal microvascular abnormality : IRMA）、数珠状静脈異常などの網膜の虚血性変化が存在する病期を非増殖網膜症のなかでも重症とし“前増殖網膜症”と位置づけた。“前増殖網膜症”はその名のとおりに増殖網膜症の前段階と考えれば重要な時期であり、現在臨床的には汎網膜光凝固の必要な時期として広く受け入れられている。この考え方は、その後の網膜症の分類に大きな影響を持ち、後述する ETDRS 分類などにも受け継がれている。

2 福田分類（表 2）

本邦では、1970 年に福田らによって分類が作成され幾度の改訂を重ね、現在では新福田分類として知られている²⁾。この分類は網膜症を中心に重症度、合併症や治療の経過を付記して表すことができるもので、本邦では現在臨床的には最も多く用いられていると思われる。網膜症を大きく良性網膜症（A）と悪性網膜症（B）とに分け、悪性網膜症には活動性の高い網膜症や治療の必要のある網膜症が含まれている。臨床的には“悪性網膜症”が“前増殖網膜症”から始まっており、この病期が光凝固治療など治療の必要な病期と考えると理解しやすい。また、増殖網膜症であっても沈静化し活動性の低い網膜症なども含まれており临床上便利である。前増殖網膜症についての考え方については、Davis 分類が前増殖網膜症を非増殖網膜症重症と捉えているのと異なり、活動性のある増殖網膜症の始まりとして捉えているなど、若干の考え方の違いが見える。

5. 糖尿病網膜症の重症度分類

表 1 糖尿病網膜症の Davis 分類

病 型	臨床所見
非増殖網膜症	
軽症網膜症 (無症状)	壁の薄い毛細血管瘤, 点状網膜出血
中等症網膜症 (黄斑浮腫がみられる場合には, 症状あり)	壁が薄いまたは厚い毛細血管瘤, 網膜出血, 硬性白斑 (輪状, 散在性), 網膜浮腫, 特に黄斑浮腫
重症網膜症 (前増殖網膜症)	網膜出血, 毛細血管瘤, 軟性白斑, IRMA, 数珠状静脈異常
増殖網膜症	
活動性の高い網膜症 (漏出性, 充血, 活動性, 代償不全)	<p>顕著な網膜所見: 網膜出血, IRMA, 数珠状静脈異常, 軟性白斑, 網膜浮腫</p> <p>新生血管: 裸の新生血管, 小さな線維増殖, 口径拡大, 乳頭近傍を含む, 急速な進展</p> <p>硝子体: 初期には収縮なし, 収縮による硝子体出血</p> <p>経過: 急速に進展, 安定期や非漏出性へ</p>
中等度の網膜症 (乾性, 静止性, 安定性)	<p>顕著でない網膜所見</p> <p>新生血管: 裸の新生血管, さまざまな程度の線維増殖, しばしば長く糸状, 乳頭近傍を含まない, 進展や寛解は緩徐</p> <p>経過: 徐々に進展, 安定期または寛解期へ</p>
燃えつきた網膜症	<p>網膜所見: 動脈狭細化・白線化・混濁, 静脈白線化・不規則少数の出血, 白斑, IRMA</p> <p>新生血管: 線維増殖膜による被覆, 消失</p> <p>硝子体: 完全収縮, 下方に陳旧性硝子体混濁</p> <p>経過: 鎮静化, ときに新鮮な硝子体出血</p> <p>網膜機能: 局所性またはびまん性の牽引性網膜剥離, 後極部が非剥離, 0.1 ~ 0.6, 重症な網膜虚血, 重篤な視力障害の原因となる</p>

IRMA: intraretinal microvascular abnormality (網膜内細小血管異常)

堀 貞夫 (編): 糖尿病眼科学 一日一課, 68, メディカル実出版, 1996 より

糖尿病眼手帳

平成 13 年に日本糖尿病眼学会は内科と眼科との連携, 情報交換などを目的とし“糖尿病眼手帳”を作成した (図 1)。この糖尿病眼手帳の中では網膜症の表現としては Davis 分類と福田分類を用いて網膜症の病期, 重症度を表記するものとなっている。

図 1 糖尿病眼手帳

日本糖尿病眼学会から入手可能である。



表2 糖尿病網膜症の新福田分類

1. 良性網膜症

1) 単純網膜症 (SDR)

a) 軽症単純網膜症 (AI)

毛細血管瘤または点状出血 (少数の点状硬性白斑)

b) 重症単純網膜症 (AII)

しみ状出血 (硬性白斑, 小軟性白斑)

2) 増殖停止網膜症 (IPDR)

a) 軽症増殖停止網膜症 (AIII)

陳旧性の新生血管 (周囲に網膜浮腫, 軟性白斑, 出血がなく6カ月以上進行を停止しているもの)

b) 重症増殖停止網膜症 (AIV, AV)

陳旧性の増殖網膜症 (6カ月以上進行なし), 硝子体出血の残るものをAIV, 増殖組織のみのものをAVとする

2. 悪性網膜症

1) 軽症悪性網膜症

a) 前増殖網膜症 (PPDR : BI)

明らかな活動性病変 (IRMA, 軟性自斑, 網膜浮腫, 線状または火焰状出血, 静脈の著明拡張) のいくつかを共存するもの

b) 早期増殖網膜症 (EPDR : BII)

乳頭に直接連絡しない新生血管 (NVE : 検眼鏡的に増殖組織なし)

2) 重症悪性網膜症

a) 中期増殖網膜症 (MPDR : BIII)

乳頭に直接連絡する新生血管 (NVD : 検眼鏡的に増殖組織なし) または乳頭浮腫を伴う後極部網膜のびまん性浮腫

b) 晩期増殖網膜症 (FPDR : BIV, BV)

硝子体腔の変化が強く加味されたもので, 単純な硝子体出血または網膜前出血を示すものをBIV, 明らかな増殖組織を伴うものをBVとする

3. 合併症

1) 黄斑病変 (M)

2) 牽引性網膜剥離 (VI または D)

3) 血管新生緑内障 (G)

4) 虚血性視神経症 (N)

*付加記号として, 光凝固による停止例には (P), 硝子体手術による停止例には (Vit) をつける。

3 Early Treatment Diabetic Retinopathy Study (ETDRS) 分類

ETDRS は網膜症に対する網膜光凝固の効果やアスピリン内服治療の効果を検討した大規模多施設無作為試験である。その研究において網膜症の客観的評価のために用いられた方法が眼底の7方向ステレオ眼底写真 (図2) を撮影し, あらかじめ定められた基準写真と比べることで重症度を判定する方法であった (以下, ETDRS 方式)。ETDRS 方式には厳密なプロトコールが存在している³⁾。

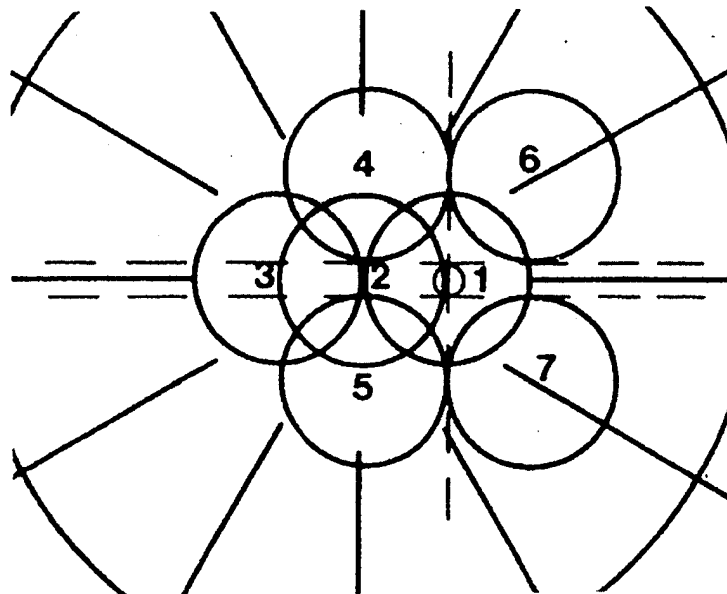


図2 ETDRS方式の写真撮影方向

眼底の7方向をステレオ写真として記録する。

Grading diabetic retinopathy from stereoscopic color fundus photographs — an extension of the modified Airlie house classification. ETDRS report #10. Ophthalmology 98:787, 1991 より

a ETDRS方式の流れ

1. 写真撮影者は眼底の撮影方法の認定を受ける。撮影方法には眼底の定められた7方向のステレオ写真が必要であり、その方法に習熟していることが求められる。
2. 認定を受けた写真撮影者によって撮影された眼底写真を Wisconsin 大学 Fundus Reading Center などの専門の判定機関に送る。
3. 判定機関では送られた写真を2人の判定者が基準写真と比較しながら所見の有無、所見の数などを判定する。判定者は判定の前に十分な訓練を受ける必要があるほか、他の判定者との診断一致率も検討される。

このようなプロセスで判定された結果から、網膜症を軽症から重症までのレベルとして重症度が表される。ただし、ETDRS分類上でのレベルには定量的な意味はなく、順序を表しているだけであることには注意が必要である。この重症度レベルを一直線上に並べたものが、ETDRS Final Retinopathy Severity Scale（一般にETDRS分類）として知られている（表3）。ETDRS方式は網膜症を客観的に高い再現性を持って判定できるシステムであり、網膜症の治療の開発に大きな基礎を与えているといえる。それはETDRS以降もETDRS方式、ETDRS分類が網膜症の客観的判定方法として、薬物をはじめとする介入試験の治療効果判定に用いられており、網膜症判定のゴールドスタンダードとなっていることに表れている。また、ETDRS方式の眼底写真撮影法を簡便化した方法（写真撮影方向を4方向にする、ステレオ写真を用いないなど）を用いて行われた研究も多くみられる（United Kingdom Prospective Diabetes Study : UKPDS⁵⁾、EURODIAB⁶⁾など）。

表3 ETDRS 分類

レベル	病 期	眼底所見
10	網膜症なし	毛細血管瘤や他の所見がみられない
20	毛細血管瘤のみ	明らかな毛細血管瘤はあるが、他の所見はない
35	軽症非増殖網膜症	以下の所見が1つ以上存在する 静脈のループ形成 \geq D/1 軟性白斑, IRMA または数珠状静脈異常 = Q 網膜出血が存在 硬性白斑, 軟性白斑 \geq D/1
43	中等度非増殖網膜症	出血, 毛細血管瘤 = M/4-5 ~ S/1, IRMA = D/1-3 (両者とも存在する必要はない)
47	重・中等度非増殖網膜症	43の所見および/または下記の所見が1つ以上存在する IRMA = D/4-5, 出血, 毛細血管瘤 = S/2-3 数珠状静脈異常 = D/1
53	重症非増殖網膜症	下記の所見が1つ以上存在する 47の所見のうち、3分の2以上がみられる 出血・毛細血管瘤 \geq S/4-5, IRMA \geq M/1 数珠状静脈異常 \geq D/2-3
61	軽症増殖網膜症	NVD, NVE を伴わない増殖膜が存在 NVE = D
65	中等度増殖網膜症	以下の所見のいずれかを伴う (1) NVE \geq M/1, NVD = D 硝子体出血および網膜前出血 = A または Q (2) 硝子体出血または網膜前出血 = D NVE < M/1, NVD は存在しない
71	危険な増殖網膜症	以下のいずれかの所見を伴う (1) 硝子体出血, 網膜前出血 \geq M/1 (2) NVE \geq M/1 および硝子体出血・網膜前出血 \geq D/1 (3) NVD = 2 および硝子体出血・網膜前出血 \geq D/1 (4) NVD \geq M
75	危険な増殖網膜症	NVD \geq M および硝子体出血・網膜前出血 \geq D/1
81	進展した増殖網膜症 部分的に透見不能 黄斑剥離なし	分類不能な NVD, または NVD < D および分類不能な NVE 黄斑の中心部の剥離 < D
85	進展した増殖網膜症 後極部の透見不能 黄斑剥離あり	硝子体出血 = 1 または 2 領域に VS 黄斑の中心部の剥離 : D
90	81 または 85 の所見がみられる が、分類不能なもの	

IRMA : 網膜内細小血管異常, NVD : 乳頭上新生血管, NVE : 網膜新生血管

A : 所見なし, Q : 疑い, D : 明らかに存在, M : 中等度存在, S : 重症, VS : 非常に重症, 1-5 : 各所見の眼底に分布する部位を示す.

堀 貞夫 (編) : 糖尿病眼科学 一日一課, 72, メディカル実出版, 1996 より

b clinically significant macular edema (CSME)

ETDRS においては黄斑浮腫に対する局所光凝固の効果を検討しているが、その際に、糖尿病黄斑浮腫を CSME という概念で分類し、治療の対象となる黄斑浮腫を設定した⁷⁾。CSME には以下の3形態がある (図3)。

1. 中心窩を含む、もしくは中心窩から $500\mu\text{m}$ 以内の網膜肥厚 (図3-A)
2. 中心窩を含む、もしくは中心窩から $500\mu\text{m}$ 以内にある硬性白斑で網膜浮腫に関連しているもの (図3-B)。
3. 1乳頭面積以上の網膜肥厚が中心窩から1乳頭径 ($1,500\mu\text{m}$) 以内にあるもの (図3-C)

判定にあたっては、ステレオ眼底写真かコンタクトレンズを用いた眼底検査により得られた所見をもとに判断するとされている。実際の治療にあたっては眼底検査に加え可能な限りフルオレセイン蛍光眼底造影を行い、“treatable lesions (治療可能な病変)”を確認した上で治療を行うことが勧められている。ここで治療可能な病変とされているのは以下のものである。

1. 中心窩から $500\mu\text{m}$ 以上離れている境界明瞭な網膜の過蛍光点 (毛細血管瘤と思われる病変) で網膜浮腫や硬性白斑の原因となっているもの
2. 中心窩から $300 \sim 500\mu\text{m}$ にあり網膜浮腫や硬性白斑の原因になっている漏出領域 (以前の光凝固治療後も残存しているか視力が $20/40$ より悪いのか中心窩の毛細血管網を破壊してしまうことがないと思われる場合)

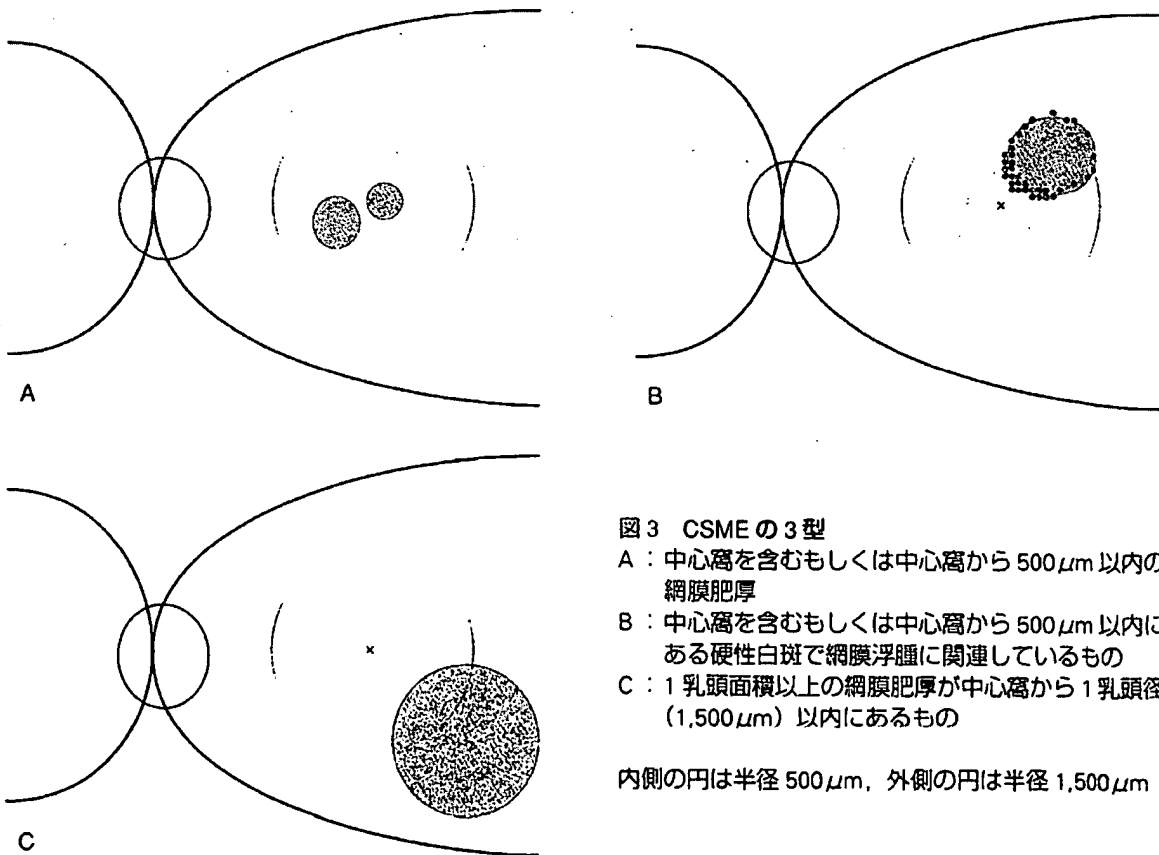


図3 CSMEの3型

- A : 中心窩を含むもしくは中心窩から $500\mu\text{m}$ 以内の網膜肥厚
 B : 中心窩を含むもしくは中心窩から $500\mu\text{m}$ 以内にある硬性白斑で網膜浮腫に関連しているもの
 C : 1乳頭面積以上の網膜肥厚が中心窩から1乳頭径 ($1,500\mu\text{m}$) 以内にあるもの

内側の円は半径 $500\mu\text{m}$ 、外側の円は半径 $1,500\mu\text{m}$

3. 局所的な蛍光漏出・蛍光漏出領域（毛細血管瘤，IRMA，びまん性の漏出）
4. 正常者にみられる中心窩無血管領域以外の網膜無灌流領域
- c. “薬物治験などに関する糖尿病網膜症判定基準”

本邦における臨床研究や治験のための重症度分類としては、2000年に日本糖尿病眼学会網膜症判定基準作成小委員会により、眼底写真を用いた“薬物治験などに関する糖尿病網膜症判定基準”が提案された⁹⁾。これは主に軽症の網膜症を対象としたもので、画角50度の眼底写真4方向を撮影しあらかじめ定めた基準写真と比較し判定を行うものである。今後は本邦においても網膜症の治療や臨床研究にあつては、このような方式やETDRS方式を網膜症の重症度の客観的評価として積極的に取り入れ、発展させていくことが重要と思われる。

4 International clinical diabetic retinopathy/macular edema disease severity scale

国際網膜症重症度分類¹⁰⁾

ETDRS分類は、ETDRS方式を使用した臨床研究などにおいては網膜症の重症度を客観的に高い再現性をもって決定できるものであるが、臨床の現場で利用することを目的としていないため、目の前にいる患者の網膜症の重症度を即座に決定することは不可能であった。そのため、厳密な意味でETDRSで得られたエビデンスを適用することは困難な状況であった。また世界各国それぞれには独自の網膜症の分類が存在しており、各国からの報告を比較する際には微妙なずれが生じていることもしばしばみられる問題であった。

そのような背景から、American Academy of Ophthalmology (AAO)により国際的に統一した網膜症重症度分類を作成するプロジェクトがつけられた。このプロジェクトはETDRS分類をもとに15カ国30人の眼科医、内科医による委員会のコンセンサスをDelphi改法により求めた結果を基にしている。その成果は平成14年10月、AAO/PAAO joint meeting spotlight sessionにおいて“Achieving consensus on an international clinical classification for diabetic retinopathy”として網膜症および黄斑浮腫の分類が報告された（表4）。この国際分類はETDRSとWisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR)から得られたエビデンスをもとに“より重症の網膜症へ進展する危険性、重篤な視力障害を来す可能性のある重症糖尿病網膜症への進行の危険性の高さ”という観点から重症度が決められており、一般眼科医、網膜疾患専門医、糖尿病専門内科医、一般内科医の間での情報交換に利用されることを目的としている。“より重症の網膜症へ進展する危険性、重篤な視力障害を来す可能性のある重症糖尿病網膜症への進行の危険性の高さ”の具体的なリスクは表4のとおりとなっている。このような具体的なリスクを踏まえた重症度分類はこれまでにないものであり、非常に説得力のあるものとなっている。

糖尿病黄斑浮腫の国際分類

国際分類では網膜症の分類のほかに黄斑浮腫を中心とした黄斑症についての重症度分類も報告した（表5）。まず糖尿病黄斑浮腫が存在するかしないかを分類し、黄斑浮腫が存在する場合にはさらに“軽症”、“中等症”、“重症”の3段階に重症度を分類する。この重症度分類はCSMEのように具体的な数値や面積を表記しておらず、網膜肥厚や硬性白斑が

表 4 糖尿病網膜症の国際分類

INTERNATIONAL CLINICAL DIABETIC RETINOPATHY DISEASE SEVERITY SCALE

Proposed Disease Severity Level 重症度分類	Findings Observable upon Dilated Ophthalmoscopy 散瞳下眼底検査所見	Derivation from ETDRS Levels 対応する ETDRS レベル	Risk Assessment リスク	Management Options* 治療の目安
No apparent Retinopathy 明らかな網膜症なし	No abnormalities 異常所見なし	Levels 10: DR absent レベル 10: 網膜症なし		Optimizing medical therapy of glucose, blood pressure and lipids 血糖、血圧、脂質異常の適正化
Mild Non-Proliferative Diabetic Retinopathy 軽症非増殖糖尿病網膜症	Microaneurysms only 網膜毛細血管瘤のみ	Levels 20: Very mild NPDR レベル 20: 軽症非増殖糖尿病網膜症		Optimizing medical therapy of glucose, blood pressure and lipids 血糖、血圧、脂質異常の適正化
Moderate Non-Proliferative Diabetic Retinopathy 中等症非増殖糖尿病網膜症	More than just microaneurysms but less than Severe NPDR 毛細血管瘤以上の病変がみられるが重 症非増殖糖尿病網膜症よりも軽症	Levels 35, 43: moderate NPDR less than 4:2:1 レベル 35, 43: 中等症非増殖糖尿病網膜症で 4:2:1 の原則以下	One year early PDR: 5.4-11.9% One year high risk PDR: 1.2-3.6% 1年後に早期増殖糖尿病網膜症に進展 する割合: 5.4-11.9% 1年後にハイリスク増殖糖尿病網膜症 に進展する割合: 1.2-3.6%	Refer to an ophthalmologist Optimizing medical therapy of glucose, blood pressure and lipids 眼科医への紹介 血糖、血圧、脂質異常の適正化
Severe Non-Proliferative Diabetic Retinopathy 重症非増殖糖尿病網膜症	Any of the following: 1) More than 20 intraretinal hemorrhages in each of 4 quadrants 2) Definite venous beading in 2+ quadrants 3) Prominent IRMA in 1+ quadrant And no signs of proliferative retinopathy	Level 47: moderate NPDR less than 4:2:1 レベル 47: 中等症非増殖糖尿病網膜症で 4:2:1 の原則以下	One year early PDR: 26% One year high risk PDR: 8.1% 1年後に早期増殖糖尿病網膜症に進展 する割合: 26% 1年後にハイリスク増殖糖尿病網膜症 に進展する割合: 8.1%	Refer to an ophthalmologist Optimizing medical therapy of glucose, blood pressure and lipids 眼科医への紹介 血糖、血圧、脂質異常の適正化

以下の所見のどれかを認め、かつ増殖網膜症の所見を認めないもの 1) 20 個以上の網膜内出血を眼底 4 象限で認める。 2) はっきりとした数珠状静脈を眼底 2 象限で認める。 3) 明確な網膜内最小血管異常 (IRMA) を認める。	53A-E: severe to very severe NPDR, 4:2:1 rule 53A-E: 重症～最重症非増殖糖尿病網膜症, 4:2:1 の原則	One year early PDR: 50.2% (severe NPDR) One year high risk PDR: 14.6% (severe NPDR) 45.0% (very severe NPDR) 1 年後に早期増殖糖尿病網膜症に進展する割合: 50.2% (重症非増殖糖尿病網膜症から) 1 年後にハイルスク増殖糖尿病網膜症に進展する割合: 14.6% (重症非増殖糖尿病網膜症から) 45.0% (最重症糖尿病網膜症から)	Consider scatter (panretinal) laser treatment for patients with type 2 diabetes Optimizing medical therapy of glucose, blood pressure and lipids 2 型糖尿病患者では汎網膜光凝固を考慮する。 血糖、血圧、脂質異常の適正化
Proliferative Diabetic retinopathy 増殖糖尿病網膜症 One or more of the following: 1) Neovascularization 2) Vitreous/preretinal hemorrhage 以下の所見のいずれかを認めるもの 1) 新生血管 2) 硝子体増殖前出血	Levels 61, 65, 71, 75, 81, 85 PDR, high-risk PDR, very severe or advanced PDR レベル 61, 65, 71, 75, 81, 85: 増殖糖尿病網膜症、ハイルスク増殖糖尿病網膜症、最重症増殖糖尿病網膜症 あるいは進行した増殖糖尿病網膜症	Strongly consider scatter (panretinal) laser treatment, without delay for patients with vitreous hemorrhage or neovascularization within one disc diameter of the optic nerve head Optimizing medical therapy of glucose, blood pressure and lipids 硝子体出血あるいは視神経乳頭から 1 乳頭径大以内の新生血管がみられた場合はすみやかに汎網膜光凝固を考慮する。 血糖、血圧、脂質異常の適正化	

* These management options are provided as general practice patterns of care. Individualized treatment plans will vary, based on several clinical considerations and factors, based on the patient's circumstances, risk factors, systemic condition, etc. These are many modifiers or risk factors not included in this classification, but which are important in risk of disease progression and in managing individual patients. These factors should be taken into account by the clinician in decisionmaking, and in informing the patient and primary care physician/diabetologist.

治療の目安は一般的な治療方針を述べたものであり、個々の患者の治療計画は臨床的な因子、患者の環境、危険因子、全身状態等によって異なってくる。この分類に含まれていないとも網膜症の進行や患者の治療に重要な因子や危険因子も数多く存在する。そのような因子も考慮しながら医師は意志決定を行い患者をはじめ内科医、糖尿病専門医に情報を伝える必要がある。

表 5 糖尿病黄斑症の国際分類

INTERNATIONAL CLINICAL CLASSIFICATION OF DIABETIC RETINOPATHY
SEVERTITY OF DIABETIC MACULAR EDEMA2 major levels, with subcategories for diabetic macular edema
2つのレベルに大別し、さらに再分類を行う

Proposed Classification 分類	Findings Observable Upon Dilated Ophthalmoscopy 散瞳下眼底検査所見
Diabetic Macular Edema Absent 糖尿病黄斑浮腫なし	No retinal thickening or hard exudates in posterior pole 後極部に網膜肥厚や硬性白斑なし
Diabetic Macular Edema Present 糖尿病黄斑浮腫あり	Some retinal thickening or hard exudates in posterior pole 後極部に網膜肥厚や硬性白斑あり

If diabetic macular edema is present, it can be categorized as follows :
もし糖尿病黄斑浮腫がみられる場合は以下に従って分類する :

Proposed Classification 分類	Findings Observable Upon Dilated Ophthalmoscopy * 散瞳下眼底検査所見
Diabetic Macular Edema Present 糖尿病黄斑浮腫あり	Mild Diabetic Macular Edema 軽症糖尿病黄斑浮腫
	Some retinal thickening or hard exudates in posterior pole distant from the macula 後極部に網膜肥厚や硬性白斑がみられるが黄斑部からは離れている。
	Moderate Diabetic Macular Edema 中等症糖尿病黄斑浮腫
	Retinal thickening or hard exudates approaching the center of the macula but not involving the center 網膜肥厚や硬性白斑が黄斑部の中心に近付きつつあるが中心を含まない。
	Severe Diabetic Macular Edema 重症糖尿病黄斑浮腫
	Retinal thickening or hard exudates involving the center of the macula 網膜肥厚や硬性白斑が黄斑部の中心を含んでいる。

* Hard exudates are a sign of current or previous macular edema. Diabetic macular edema is defined as retinal thickening and this requires a 3-dimensional assessment that is best performed by a dilated examination using slit-lamp biomicroscopy and/or stereo fundus photography.

硬性白斑は現在あるいは過去の黄斑浮腫の存在を示している。黄斑浮腫を網膜の肥厚と定義する場合、立体的な眼底の評価が必要であり、散瞳下に細隙灯顕微鏡を用いた検査と場合によってはステレオ眼底写真をもとに診断する。

“黄斑の中心から離れている” “黄斑の中心に近づいている” などの表現で表されている。そのため重症度の判定は使用者の臨床現場での判断に任されることになる。山形大学眼科ではETDRSでの黄斑浮腫の定義⁹⁾をもとに便宜的に以下のように解釈している(図4)。

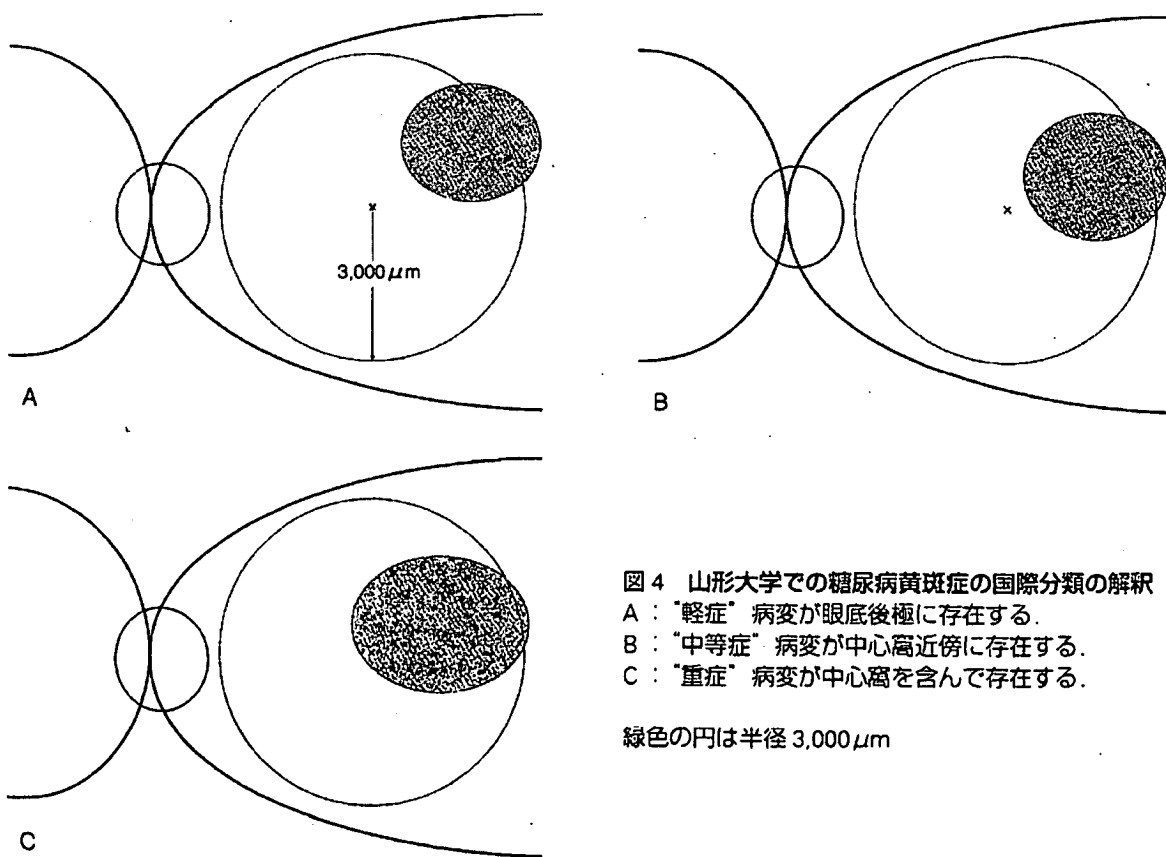
“黄斑浮腫”を中心窩を中心に半径約3,000 μ m(2乳頭径)以内にみられる網膜肥厚(網膜浮腫)や硬性白斑といった病変と中心窩の位置関係を基準として以下の3段階に分類する。

表6 各分類の比較表

糖尿病網膜症の 主な眼底所見	新福田分類	Davis 分類	ETDRS 分類	International clinical diabetic retinopathy disease severity scale
毛細血管瘤 点状出血	A I	非増殖網膜症 軽症	20	Mild non-proliferative diabetic retinopathy
しみ状出血 硬性白斑	A II	非増殖網膜症 中等症	35 43	Moderate non-proliferative diabetic retinopathy (注 1)
軟性白斑	A II ~ B I	非増殖網膜症	47	
IRMA	B I	重症 (増殖前網膜症)	53	Severe non-proliferative diabetic retinopathy (注 2)
静脈異常				
新生血管	A III, B II, B III	増殖網膜症	61, 65	Proliferative diabetic retinopathy
硝子体出血	A IV, B IV, B V		65, 71, 75, 85	
牽引性網膜剥離	VI または D		81, 85	

(注 1) 硬性白斑、軟性白斑の存在によらない。

(注 2) 毛細血管瘤が 4 象限にそれぞれ 20 個以上あれば Severe non-proliferative diabetic retinopathy とする。



“軽症”：病変が中心窩から半径 500 ~ 3,000 μm に存在する。

“中等症”：病変が中心窩から半径 500 μm 以内に存在する。

“重症”：病変が中心窩を含んで存在する。

今後この分類が用いられる機会が増えることが予想され、その有用性の検証とともに国際的に浸透し発展することが期待される。

表 6 に各分類の対比表を示す。

C 重症度分類の意義

現在、網膜症の治療としては、主に汎網膜光凝固と局所網膜光凝固および硝子体手術が行われている。これらの治療を適切な時期に行うことができれば、95% 以上で網膜症による重篤な視力障害を防ぐことができるという試算がある¹⁰⁾。

しかしながら網膜症はなお、本邦をはじめとする各国の成人における後天性失明原因の上位を占めている。その背景には網膜症が自覚障害のないまま発症し進行してしまい、眼科受診時には治療の時期を逃してしまっているケースがあると思われる。平成 14 年に山形大学眼科外来で行った調査では、眼科初診時にすでに視力障害を伴う増殖網膜症であった 50 歳以下の 2 型糖尿病患者を対象として受診までの経過を調べたところ、検診や病院受診で糖尿病と診断されてから眼科を受診するまでに平均 10 年を要していた（非増殖網膜症群は平均 3 年）。このことは網膜症の早期発見、眼科での早期の診断が重要であることを示している。同時に自己判断で通院や治療を中断してしまうケースの問題も重要である。糖尿病と診断されて早期に眼科を受診しても、軽症例では自覚症状がないため自己判断で受診を中断する例や、眼科受診時に網膜症がみられなかったため医師から「問題ありません」と説明を受け、それを二度と眼科を受診しなくともよいと言われたものと勘違いした例なども見受けられる。

このような治療中断を予防するためには、眼科医が患者に網膜症について進行性の疾患であることなどの知識を伝えと同時に、患者の眼底所見に基づき網膜症の重症度を正確に把握し、現在網膜症の中でどの程度の重症度であるかを患者に伝え、今後重篤な視力障害を来す可能性がある増殖網膜症への進展の危険性を推し量りながら、適切な時期に定期検査を行い、適切な治療時期を逃さないように努めることが肝要である。そのためには、網膜症の眼底所見を正確に捉え、眼底所見に基づき網膜症の重症度を理解し、それぞれのリスクを把握していることが重要である。患者には網膜症が病期のどの程度にあるのか、重症度はどの程度か、現在治療が必要な時期にあるのかあるいは近々治療が必要になる可能性はあるか、などの情報を伝えて認識させることも重要である。臨床的な福田分類などの病期分類や重症度分類は、そのような意味で非常に有用なものである。

新たな臨床研究や治療方法の開発にあたっては、治療効果を判定するのに網膜症の重症度を客観的に評価する方法が不可欠であり、そのような目的で作られた ETDRS 方式・ETDRS 分類などの重症度分類も発展してきた。また、ETDRS 分類を臨床に応用するための国際分類も作成され、研究の結果を臨床に応用したり、ETDRS など得られた網膜症進行のリスクを把握するのに有用であり、今後の発展が期待される。

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ONO-2235 の経口投与におけるイヌでの 3 ヶ月亜急性毒性試験および 1 ヶ月回復試験

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〔要約〕ONO-2235 の20, 100および500mg/kg をビーグル犬に90日間投与し, その毒性および回復性を検討し, 以下の如き結果を得た.

1. 一般症状の変化としては嘔吐が100mg/kg 以上の投与群, 軟便の頻度の増加が500mg/kg 投与群に観察され, 500mg/kg 投与群の雄 1 例が投与32日目に死亡した.

2. 体重の減少が100mg/kg 投与群の雌 1 例と500mg/kg 投与群の雌雄, 摂餌量の減少が500mg/kg 投与群の投与30~44日目と79日目以降 (雄のみ), 摂水量の増加が500mg/kg 投与群の16日目以降に観察された.

3. 尿検査では, 尿量の増加傾向および尿比量の減少傾向 (雄のみ) が500mg/kg 投与群に認められた.

4. 血清生化学検査および BSP 排泄試験において, 500mg/kg 投与群に GOT・GPT 活性の増加または増加傾向, アルブミン量の減少を伴う A/G 比の低下および BSP 排泄能の低下が観察された.

5. 心電図検査では100mg/kg 以上の投与群に心拍動数の減少が認められた.

6. 各臓器の肉眼的および重量的検査において, 500mg/kg 投与群に腎臓の退色腫大, 胸腺の退縮, 雄生殖器 (精巣, 精巣上体, 前立腺) の重量減少と前立腺の萎縮が認められた. 組織学的検査では500mg/kg 投与群の腎臓に尿細管上皮細胞の変性・壊死と刷子縁の脱落, 尿細管腔の拡張, 円柱の出現, 肝臓に Kupffer 細胞への hemosiderin の沈着, 胸腺の退縮, 前立腺の低形成が観察された. また, 肝臓および腎臓の電子顕微鏡検査では, いずれも機能的な適応に伴うものと考えられるが500mg/kg 投与群の肝臓でメガミトコンドリアの増加とこのミトコンドリア内にラメラボディをもつものが, 100mg/kg 以上の投与群の腎臓で近位尿細管細胞のミトコンドリア数の増加と環状あるいは縦走層板状クリステをもつミトコンドリアが認められた.

7. これらの変化は休薬により回復する可逆的な変化であった。
 8. 以上の結果より、本試験における ONO-2235 の無影響量は20mg/kg と推察される。

目 的

本実験は、糖尿病性合併症の治療剤として開発された ONO-2235 をビーグル犬に90日間毎日1回強制経口投与し、亜急性毒性とその回復性を検討するために実施した。

実験材料および方法

1. 使用動物および飼育条件

実験には入荷後1ヵ月間予備飼育し、健康に推移した6～7ヵ月齢のビーグル犬 (Hezleton Lab.産) で体重雄7.2～10.5kg, 雌7.6～10.1kg のものを雌雄各24匹使用した。動物は体重別に層別化し、平均体重が同じになるように、各群に割り付けた。

各群の動物数は1群あたり雌雄各6匹とし、そのうちの雌雄各2匹については30日間の回復試験を行った。(Appendix 1)

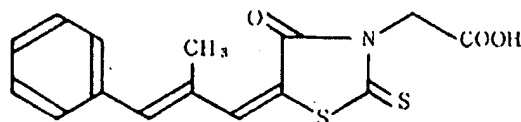
動物は室温23±2℃, 相対湿度55±10%, 照明時間12時間 (7:00～19:00), 換気回数12回/hr に調節された飼育室でアルミ製個別ケージ (85cm×75cm×60cmH) に収容し飼育した。

飼料は固形飼料 (ラボDストック, 日本農産工業) 300g を1日1回投与終了1～2時間後に、飲料水は塩素滅菌水を朝と夕方の2回1,000ml/ずつセットし、それぞれ摂取させた。

2. 被験物質

ONO-2235 は、無味、微かな特異臭のある橙黄色

結晶で下記の化学名および化学構造の化合物である。本試験には試験委託者 (小野薬品工業株式会社) より提供された Lot. J-3004 を用いた。



ONO-2235 : 5-[(1E, 2E)-2-methyl-3-phenylpropenylidene]-4-oxo-2-thioxo-3-thiazolidineacetic acid (C₁₅H₁₃NO₃S₂, M. W. 319.40)

3. 投与量および投与方法

投与に先だって、最も新しい体重に基づいた所要量をゼラチンカプセル (Na00および000, 局方, 吉田商店) に充填し, 1日1回90～91日間強制的に経口投与した。対照群は500mg/kg のバレイショデンプン (局方, 丸石製薬) をカプセルに充填し, 同様に投与した。

本試験における投与量は急性毒性試験¹⁾で500および1,000mg/kg 投与群の全動物に検体と思われる黄色便の排出と1,000mg/kg 投与群に嘔吐が観察されたため、最高量を500mg/kg とし, 以下に100および20mg/kg を設定した。

4. 観察方法

1) 一般状態の観察

実験期間中は一般症状の観察を毎日, 体重, 摂餌量および摂水量の測定を投与開始前1回と投与開始後は週1回行った。

Appendix 1. Animal No. on subchronic toxicity study of ONO-2235 in dogs

Group (mg/kg)	Male						Female					
	Termination ^{a)}				Recovery ^{b)}		Termination				Recovery	
Control	1, 3, 5, 6	2, 4	501, 504, 505, 506	502, 503								
20	7, 8, 11, 12	9, 10	507, 508, 509, 511	510, 512								
100	13, 14, 16, 18	15, 17	514, 516, 517, 518	513, 515								
500	19, 21, 22, 23	20, 24	519, 521, 522, 524	520, 523								

a) : Treatment for 90 days, b) : Withdrawal for 30 days

2) 尿 検 査

投与前2週目, 投与4および12週目, 休薬4週目の4回にわたり, 短時間の蓄尿について比重(屈折計, エルマ), pH・蛋白・糖・ketone 体・潜血・bilirubin・urobilinogen (マルティスティックス® III, Ames) および沈渣を検査するとともに, 夕方より翌朝までの16時間蓄尿について尿量, Ca^{2+} (Ca-Mg メーター, 30/20, 常光), Na^+ と K^+ (炎光光度計, 205-D, 日立) および Cl^- (Chloride counter, CL-5, 平沼) を測定した。

3) 血液学的検査

投与前5日目, 投与30および90日目, 休薬30日目の4回, 無麻酔下で前腕皮静脈より採取した EDTA-2K 加血液について赤血球数, hemoglobin 量, hematocrit 値, 網状赤血球数, 血小板数, 白血球数および白血球百分率を, sodium citrate 加血漿について prothrombin time および partial thromboplastin time を測定した。

測定項目および測定方法

赤血球数 (RBC)

: Microcell counter (CC-108, 東亜)

Hemoglobin 量 (HB)

: Hemoglobin counter (HB-100, 東亜)

Hematocrit 値 (HT): 毛細管法

網状赤血球数 (RET): Brecher 法

血小板数 (PLAT)

: Platelet counter (PL-100, 東亜)

白血球数 (WBC)

: Microcell counter (CC-108, 東亜)

白血球百分率: May-Grünwald Giemsa 染色

Prothrombin time (PT)

: クロテック (Hyland)

Partial thromboplastin time (PTT)

: クロテック (Hyland)

さらに RBC, HT および HB の値より平均赤血球恒数 (MCV, MCH, MCHC) を算出した。

4) 骨髓検査

投与終了後 (91および92日目) および休薬終了後 (休薬31日目) の解剖時に各動物の肋骨より採取した骨髓組織を濃塗抹した後, May-Grünwald

Giemsa 染色を施し鏡検に供した。

5) 血清生化学的検査

血液検査用試料採取時に同時に採血し分離した血清について, 以下の項目を測定した。

測定項目および測定方法

総蛋白質 (TP): Biuret 法*

蛋白分画および A/G 比

: Cellulose acetate 膜による電気泳動法

総 cholesterol (T-CHO): 酵素法*

Triglyceride (TG): 酵素法*

リン脂質 (PL): 酵素法*

Transaminase (GOT, GPT): UV 法*

Alkaline phosphatase (ALP): Kind king 法*

血 糖 (GLU): Glucose oxidase 法*

尿素窒素 (BUN): Urease Indophenol 変法*

Creatinine (CRE): Jaffé 法*

尿 酸 (UA): 酵素法*

総 bilirubin (T-BIL): Jendrassik Cleghorn 法*

Lactate dehydrogenase (LDH): UV 法*

Creatine phosphokinase (CPK): Oliver 法*

Amylase (Amy): ヨード澱粉比色法*

Calcium (Ca^{2+}): OCPC 法*

無機リン (P): モリブデンブルー直接法*

Na^+ , K^+ : 炎光光度計 (205D, 日立)

Cl^- : 電量滴定法

(Chloride counter, CL-5, 平沼)

以上の項目のうち*印は Automatic analyzer (706D, 日立) で測定した。

6) 肝および腎臓機能検査

投与前2週目 (PSP は1週目) および投与12週目と休薬3週目の異なる日に, 肝機能検査として heptosulphalein (BSP) 注射液 (局方, 第一製薬) の 5 mg/kg/0.1ml を, 腎機能検査として phenolsulfonphthalein (PSP) 注射液 (局方, 第一製薬) の 0.6mg/kg/0.1ml を前腕皮静脈内に注射し, その30分後に対側の同静脈より採血した EDTA-2K 加血漿中の残余 BSP および PSP 量を測定することにより, 肝臓および腎臓の色素排泄能を検査した。

7) 眼科的検査

投与前1週目、投与12週目および休薬4週目に各動物の両眼の結膜、角膜、強膜および虹彩をスリットランプ(HE-199, 半田屋商店)を用いて検査した。その後、散瞳剤(ミドリン®P, 参天製薬)を点眼後、大動物用眼底カメラ(RC-2, 興和)を用いて眼底の状態を観察するとともに、眼底の写真撮影を行った。

8) 心電図検査

心電図は眼科的検査と同時期の当日の投与前にポータブル心電計(FD-14, フクダ電子)を用いて測定した。測定時にはイヌを無麻酔下の横臥位にし、AB誘導法に従ってAB-I, 四肢誘導法に従ってI, II, III, aVR, aVL およびaVFの各波形を記録した。

9) 剖検および臓器重量検査

実験期間途中の死亡例ならびに連続投与および休薬期間終了後にsodium pentobarbital麻酔下で放血死させた各動物について、各臓器の肉眼的異常の有無を観察するとともに、各臓器の摘出を行った。生存例の摘出臓器のうち、心臓、肺、肝臓、腎臓(左右別)、脾臓、副腎(左右別)、精巣(左右別)、精巣上体、前立腺、卵巣(左右別)、子宮、胸腺、甲状腺、脳、下垂体および顎下腺についてはその湿重量を測定するとともに、体重1kgあたりの比重量を算出した。

10) 病理組織学的検査

i) 光顕

摘出した各臓器は10%中性formalin液に固定し、paraffin切片を作成後、hematoxylin eosin染色を、ほかに100mg/kg以上の投与群の腎臓についてはPAS染色を、500mg/kg投与群の1部の例の肝臓については鉄染色(Berlin blue染色)を施し、それぞれを鏡検に供した。

検査臓器は以下に示したが、そのうちで顎下腺、眼球、精巣、精巣上体、卵巣および子宮は左側のみを病理組織学的検査に供し、これら臓器の右側と聴覚器、大腿骨、胸骨、耳下腺、横隔膜、骨格筋、咽頭および肛門はformalin液中に保存した。

検査臓器(採取部位)

心臓(左室壁、右室壁、中隔)、肺(左肺、右

肺)、肝臓(外側左葉、外側右葉、胆のう部)、腎臓(左腎、右腎)、舌、気管、食道、扁桃、胃(胃底腺、幽門部)、小腸(十二指腸、空腸、回腸)、大腸(結腸、盲腸、直腸)、脾臓、顎下腺、下垂体、甲状腺、副腎(左、右)、胸腺、脾臓、顎下リンパ節、腸間膜リンパ節、胸椎骨髓、大脳(灰白質、視床、視床下部)、小脳、延髄、頸髄、坐骨神経、精巣、精巣上体、前立腺、卵巣、子宮、膣、喉頭、眼球、膀胱、大動脈(胸部、腹部)、皮膚(乳腺を含む)、その他病変部

ii) 電顕

各群の雌雄各3例(投与終了時2例、休薬終了時1例)より摘出した肝臓および腎臓(皮質)の小片を2% glutaraldehyde液および1% osmic acid液に二重固定し、epon樹脂に包埋した。

標本は常法に従い、ウルトラミクロトームで超薄切片とし、酢酸ウラニウムおよび硝酸鉛で二重染色し、電子顕微鏡(JEM-100S型、日本電子KK)で観察した。

なお、病理組織学的検査の電顕検索は小野薬品工業株式会社中央研究所にて実施した。

5. 統計手法

各測定値より、各群の平均値および標準偏差値を算出してF検定を行い、等分散の仮定が成立した場合はt検定を、成立しない場合はt検定の近似式(Aspin-welch法)を用いて、各投与群と対照群間の有意性を検定した。また投与前値との比較も合せ行った。

実験成績

1. 一般状態の観察

1) 一般症状

100mg/kg以上の投与群に黄色便、嘔吐および一般状態の悪化、500mg/kg投与群に流涎および血便が認められた。しかし流涎および血便の発現頻度は低く、経時的な相関性のないものであった。一般状態の悪化は100mg/kg投与群の雌1例(Na513)の78日目、500mg/kg投与群の雄1例(Na21)で28日目と他の1例(Na19)で76日目、同群の雌3例(Na519, 520, 522)で投与78~79日

目より認められ、そのうち最も体重減少の強かった雄1例 (Na21) が投与32日目に死亡した。

その他、対照群を含む全群で軟便が観察され、500mg/kg 投与群で、その発現頻度が若干多い傾向を示した。

休薬群では、一般状態の悪化の認められた例も回復または回復の傾向が認められた。

2) 体 重

各群の体重の推移を図1に示した。

100mg/kg 投与群の雌1例 (Na513) で投与29日目、500mg/kg 投与群の雌雄で投与22日目より体重の減少傾向がみられ、以後減少または横這い状態が継続し、各群の平均体重は、500mg/kg 投与群の雄で投与57日目、雌で29日目より対照群との間に有意差がみられた。

休薬群では500mg/kg 投与群の雌雄とも体重の減少は順調に回復したが、対照群値までには至らなかった。

3) 摂餌量および摂水量

各群の摂餌量は図2に、摂水量は図3に示した。

摂餌量では雌雄とも100mg/kg 以下の投与群に特記すべき変化は認められなかったが、500mg/kg 投与群で雄1例 (Na21) に投与16日目より、他の1例 (Na19) に51日目より、雌の1例 (Na519) に投与23～51日目に減少がみられ、平均摂餌量も当該群の雄で投与30～37日と79日目以降、雌で投与30～44日に軽度減少した。しかし、休薬群に特記すべき変化はみられなかった。

摂水量では500mg/kg 投与群の雄4例 (Na19, 20, 22, 24) および雌2例 (Na520, 522) に投与16日目頃より、20mg/kg 投与群の雌1例 (Na509) に投与58日目頃より増加がみられ、平均摂水量も同群の同時期に増加を示した。休薬群の摂水量は500mg/kg 投与群の雌雄で対照群に比し若干多い傾向を示した。

2. 尿 検 査

各群の尿量、尿比重および定性検査の結果は表1～9に、尿電解質の結果は表10～17に、尿沈渣の結果は表18～26に示した。

尿量および尿比重の検査では500mg/kg 投与群の雌雄に投与期間および休薬期間を通じて尿量の

増加傾向と雄にはさらに尿比重の減少傾向が認められた。

定性検査では、尿糖、ketone 体、潜血の陽性とbilirubinの増加が認められ、尿糖の陽性 (いずれも±) は100mg/kg 投与群の4週目に雄3例 (Na14, 15, 16) と雌1例 (Na517)、500mg/kg 投与群の4週目に雌雄各3例 (Na19, 21, 23およびNa519, 520, 523) と12週目に雄1例 (Na19) に認められたが、経時的な相関性はなかった。

Ketone 体の陽性 (いずれも+) は100mg/kg 投与群の4週目に雄3例 (Na14, 15, 16) と雌1例 (Na517) および12週目に雄2例 (Na15, 17)、500mg/kg 投与群の4週目に雄3例 (Na19, 21, 23) と雌4例 (Na519, 520, 522, 523) および12週目に雄2例 (Na19, 23) と雌4例 (4週目と同じ例) にみられ、潜血陽性は20mg/kg 投与群の雌1例 (Na510, ±) と100mg/kg 投与群の雌雄各1例 (Na17, ±; Na514, ++) の12週目、500mg/kg 投与群の4週目に雌雄の各2例 (Na21, +++; Na23, +; Na519および521, ±) と12週目に雄1例 (Na22, ±) および雌3例 (Na519, 521, ±; Na520, +) に観察された。Bilirubinの増加 (いずれも+) は500mg/kg 投与群の雄2例 (Na21, 23) および雌1例 (Na520) の4週目に認められたが、12週目には陽性例なく、経時的な相関性を欠いていた。

尿電解質の検査では100mg/kg 以下の投与群に変化はみられなかったが、500mg/kg 投与群で、4週目に雄でCa²⁺値の増加と雌でK⁺値の低下を認めたのみで、経時的な相関性は認められなかった。

尿沈渣では、赤血球の出現 (Na21の+以外はいずれも±) が、4週目で対照群の雄1例 (Na1), 20mg/kg 投与群の雄2例、雌1例 (Na8, 11, 511), 100mg/kg 投与群の雌雄各1例 (Na14, 518), 500mg/kg 投与群の雄2例雌3例 (Na21, 23, 519, 521, 524) に、12週目で対照群雌1例 (Na505), 20mg/kg 投与群雌1例 (Na508), 100mg/kg 投与群雄1例 (Na18) および500mg/kg 投与群の雄1例 (Na23) に認められた。しかし、経時的な相関性を示した例は500mg/kg 投与群の雄1例 (Na23) のみであった。

ほかに上皮細胞、リン酸アンモニア、マグネシ

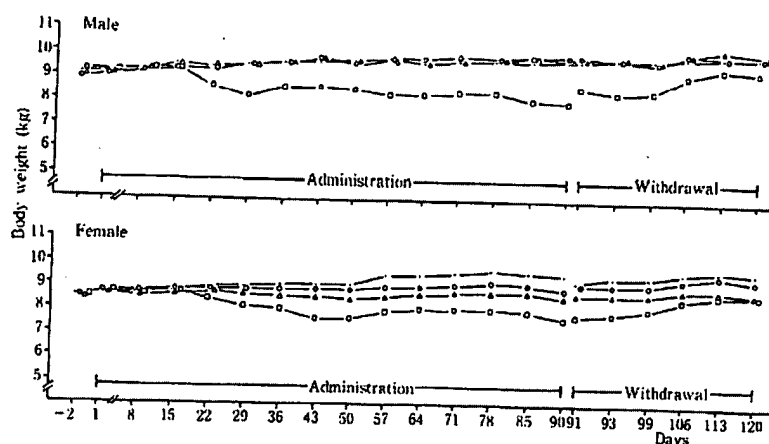


图 1. Changes of average body weight in dogs treated orally with ONO-2235 for 90 days
●—● Control ○—○ 20mg/kg △—△ 100mg/kg □—□ 500mg/kg

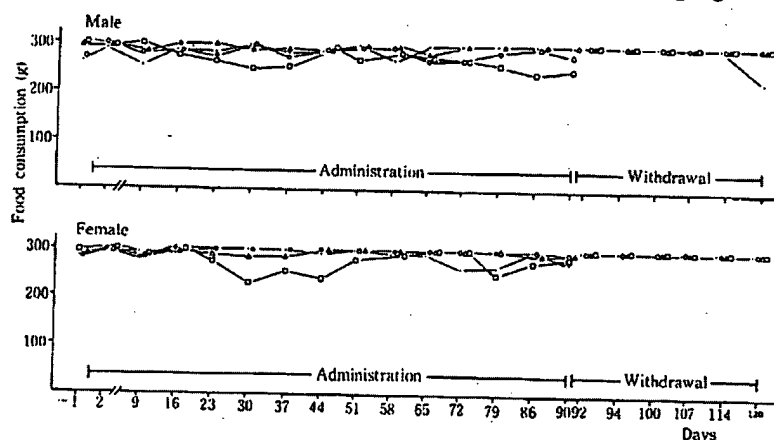


图 2. Changes of average food consumption in dogs treated orally with ONO-2235 for 90 days
●—● Control ○—○ 20mg/kg △—△ 100mg/kg □—□ 500mg/kg

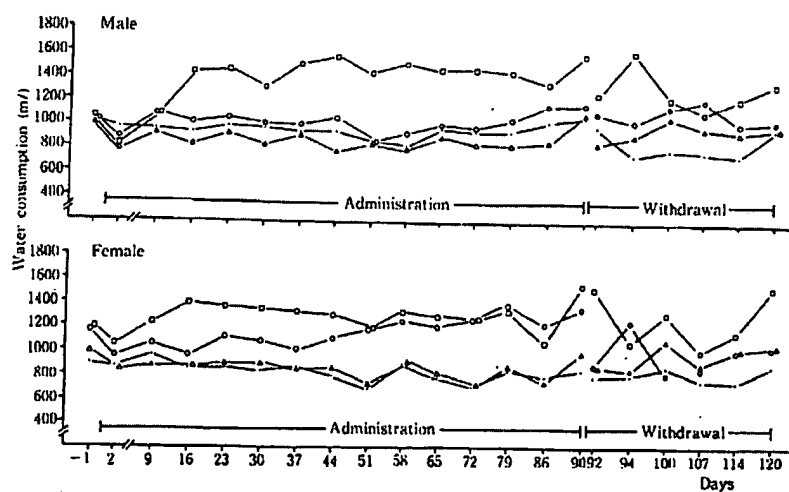


图 3. Changes of average water consumption in dogs treated orally with ONO-2235 for 90 days
●—● Control ○—○ 20mg/kg △—△ 100mg/kg □—□ 500mg/kg

ア結晶および円柱などが認められたが、その出現率に各群間に差異はみられなかった。

休薬例では20mg/kg 投与群の雌1例(Na512)で潜血反応の陽性(卅)と尿沈渣に赤血球の出現(±)、500mg/kg 投与群の雌1例(Na523)に潜血反応陽性(+)が認められたが、この2例は、4および12週目の検査で陰性を示しており、検体投与とは関連性のない変化と考えられた。また尿電解質にも特記すべき変化はみられなかった。

3. 血液学的検査

各群の検査結果を表27~28に示した。

雄の20mg/kg 投与群の1ヵ月目に赤血球数、hematocrit 値、3ヵ月目に hematocrit 値の低下がみられたが、この変動は投与前にも認められており、検体投与に起因した変化ではなかった。ほかに20mg/kg 投与群の雄に単球数の減少およびPTTの短縮と雌にMCHの増加(いずれも1ヵ月目)、100mg/kg 投与群にリンパ球数の増加と単球数の減少(1ヵ月目)およびPTTの短縮(3ヵ月目)、500mg/kg 投与群の雄に血小板数(3ヵ月目)およびMCHの増加(1ヵ月目)、雌でhemoglobin 量、MCH および MCHC の増加(いずれも1ヵ月目)が認められたが、これらの変化に用量および経時的な相関性はみられず、正常範囲を逸脱する個体も観察されなかった。

休薬例に特記すべき変化は認められなかった。

4. 骨髄検査

各群の検査結果を表29~33に示した。

雌雄共に用量相関性を示す著明な変動は認められなかった。

5. 血清生化学検査

各群の検査結果を表34~35に示した。

500mg/kg 投与群のほぼ全例の1および3ヵ月目にGOT および GPT 活性の増加、雌雄各3例(Na19, 20, 24, 519, 522, 523)の3ヵ月目にアルブミン量の減少を伴うA/G比の低下がみられ、同群の平均値でもGOT および GPT 活性の増加または増加傾向、アルブミン量の減少ならびにA/G比の低下が認められた。

その他の項目においても、変動を示すものが散見されたが、投与前値と大差なく、また用量相関

性も認められず、検体投与に起因した変化とは考え難いものであった。

休薬群では、500mg/kg 投与群の GOT および GPT 活性、アルブミン量およびA/G比はいずれも正常に復した。

6. BSP および PSP 排泄試験

各群の検査結果を表36~37に示した。

BSP 排泄試験において、500mg/kg 投与群の雌雄の各4例(Na19, 20, 22, 23およびNa519, 520, 522, 523)に血中BSP量の増加がみられ、BSP排泄能が低下したが、100mg/kg以下の投与群に変化は認められなかった。この変動は休薬により正常に復した。

PSP 排泄試験では検体投与に起因した異常所見は観察されなかった。

7. 眼科的検査

各群の雌雄とも角膜、結膜、強膜、虹彩および眼底の血管などに検体投与に起因した異常所見は認められなかった。

8. 心電図検査

雌雄とも100mg/kg以上の投与群で投与12週目に心拍動数の減少傾向がみられ、徐脈となる傾向を示した。しかし、この心拍動数の減少は休薬により回復した。

ほかに、T波の増高、洞ブロック、上室性または心室性の期外収縮、呼吸性不整脈、軽度な僧帽性または肺性P波、軽度なST部分の降下などの所見が対照群を含む各群の数例にみられたが、これらの変化は投与前にも認められるものであると同時に用量相関性もみられないことより検体投与に起因する変化とは考え難かった。

9. 剖 検

500mg/kg 投与群にみられた死亡例(Na21)では大動脈、腎盂部、膀胱および胆嚢内面の黄染と精巣、精巣上体および前立腺の萎縮が観察された。

生存例では500mg/kg 投与群の雌雄の各3例(Na19, 22, 23およびNa519, 521, 522)に腎臓の退色腫大(写真1)がみられ、これらの例のうち、雄の1例(Na23)を除く他の例に胸腺の萎縮、Na19に胃粘膜の白斑、Na22に前立腺の萎縮が観察された。ほかに対照群の雌1例(Na504)の胃噴門部に

点状出血が認められた。

休薬群では500mg/kg 投与群の雄1例(Na24)に腎臓の退色がみられたが、投与終了時に認められた腫大は観察されなかった。

ほかに20mg/kg 投与群の雌1例(Na512)に胸腺の萎縮が認められた。

10. 臓器重量

各群の検査成績を表38～45に示した。

湿重量および比重量に共通した変化としては500mg/kg 投与群の雌雄全例に腎臓重量の増加または増加傾向と胸腺重量の減少傾向が認められた。また500mg/kg 投与群の雄に精巣、精巣上体および前立腺の湿重量の減少と前立腺の比重量の減少傾向が認められた。

ほかに湿重量で20mg/kg 投与群の雄に甲状腺重量の増加と雌に下垂体重量の減少が認められたが、これらの変動は極く軽度で用量相関性もみられないことより検体投与に起因した変化とは考え難かった。

休薬群では500mg/kg 投与群の雌雄にみられた腎臓重量の増加および胸腺重量の減少傾向、雄にみられた精巣、精巣上体および前立腺の湿重量の減少傾向はほぼ正常に復した。

11. 病理組織学的検査

1) 光 顕

各群の検査結果を表46～47に示した。

検体投与に起因したと考えられる変化が腎臓、肝臓、胸腺および前立腺に認められた。

i) 腎 臓

500mg/kg 投与群の死亡例を含む雌雄の全例に主部尿細管上皮細胞の変性および壊死、刷子縁の脱落がみられ(写真2)、それらのうちの雄3例(Na19, 21, 23)、雌1～3例(Na502とNa519, 512, 522)に尿細管の拡張と円柱の出現が観察された。しかしこれらの変化はいずれも極く軽度～軽度なものであった。

ほかに、主部尿細管直部の上皮細胞に空胞変性が対照群の雄2例(Na3, 5)、20mg/kg 投与群の雌3例(Na507, 508, 511)、集合管の拡張が20mg/kg 投与群の雌1例(Na507)および腎盂部への細胞浸潤が500mg/kg 投与群の雌1例(Na519)にそれ

ぞれ認められたほかに、対照群の2例の当該例ではさらに尿細管腔に円柱の出現も観察されたが、これらの変化はビーグル犬にしばしばみられ、用量相関性も認められないことより検体投与に起因したものと考え難かった。

休薬群では20mg/kg 投与群の雌2例(Na510, 512)および100mg/kg 投与群の雌1例(Na515)で主部尿細管直部の上皮細胞に空胞変性、100mg/kg 投与群の雄1例(Na15)に泡沫細胞を伴う小結節が認められた。

ii) 肝 臓

500mg/kg 投与群の雄4例(Na19, 21(死亡), 22, 23)および雌1例(Na521)のKupffer細胞に軽度の褐色色素の沈着が認められたが、この色素は鉄反応陽性であった(写真3)。また死亡例には小葉中心性のうっ血が観察された。

ほかに、肝細胞の淡明化が対照群、100および500mg/kg 投与群の雄各1例(Na6, 18, 20)に、グリソン氏鞘部への細胞浸潤が対照群および500mg/kg 投与群の雌(Na506, 521)、20mg/kg 投与群の雄(Na7)の各1例に認められたが、これらの変化に用量相関性はみられなかった。

休薬群では500mg/kg 投与群の雌雄の1例(Na24, 520)にKupffer細胞への褐色色素の沈着、他の雄1例(Na20)に肝細胞の淡明化が認められたが、他の群に変化は観察されなかった。

iii) 胸 腺

胸腺の退縮が500mg/kg 投与群で雄の3例(Na19, 21(死亡), 22)および雌の全例、20mg/kg 投与群で雌雄の各2例(Na7, 12; Na507, 508)、対照群で雄の1例(Na5)に観察されたが、その退縮像は500mg/kg 投与群で強い傾向を示した。

休薬群では20および100mg/kg 投与群の雌各1例(Na512, Na513)に胸腺の退縮が認められたが、500mg/kg 投与群には観察されず、明らかな回復がみられた。

iv) 前 立 腺

500mg/kg 投与群の3例(Na19, 21(死亡), 23)に前立腺の低形成が認められた。

その他の臓器においても種々の変化が観察されたが、用量相関性を示すものはなかった。

2) 電 顕

i) 腎 臓 (写真4,5)

20mg/kg 投与群には特記すべき異常はなかった。100および500mg/kg 投与群雌雄の近位尿細管上皮細胞で、ミトコンドリア数の増加傾向が、またミトコンドリアにおいて長軸方向に縦走する層板状のクリステを有するもの、近位尿細管上皮および管腔内細胞成分中に、環状あるいは同心円状のミトコンドリアが認められた。しかし、近位尿細管上皮においても他のオルガネラに異常はなく、また腎臓(皮質)のその他の部位にも異常はなかった。

休薬後の例では前述したようなミトコンドリアの変化はなく、その他の部位にも全く異常は認められなかった。

ii) 肝 臓 (写真6,7)

500mg/kg 投与群の肝細胞で内部に高電子密度のラメラボディーをもつメガミトコンドリアが認められた。また、光顕所見と同様にKupffer細胞中にヘモジデリンと思われる顆粒が認められた。しかし肝細胞のその他のオルガネラおよび肝臓のその他の部位における異常はなかった。

休薬例では、500mg/kg 投与群の雌1例にメガミトコンドリアが認められたが、その他の例には異常はなく、回復の傾向が伺えた。

考 察

ONO-2235 投与群にみられた異常所見は、100mg/kg 以上の投与群における嘔吐、一般状態の悪化、体重の減少、ケトン体および潜血の陽性化、心拍動数の減少、500mg/kg 投与群における死亡の発現、摂餌量、摂水量、尿量、尿比重、GOT、GPT 活性ならびに BSP 排泄量の変動ならびに腎臓、肝臓、胸腺および前立腺の重量的または形態的な変化であった。

しかし、ケトン体の陽性化は、ONO-2235 の代謝物を尿に添加した場合に認められることより、尿中への代謝物の排泄によるものと考えられる。また潜血反応については、明らかな陽性例は4週目の500mg/kg 投与群の雄2例(1例は約1週後に死亡)、12週目の100mg/kg 投与群の雌1例、500

mg/kg 投与群の雌1例に認められ、いずれも異なった個体に認められ経時的な相関性がないことより検体投与に起因するものとは考え難い。また100mg/kg 以上の投与群で黄色便がみられたが、ONO-2235 あるいはその代謝物によるものと考えられた。一方、嘔吐、一般状態の悪化、体重減少、摂餌量の減少、死亡例の発現は、ONO-2235 投与に起因したものと考えられ、500mg/kg 投与群にみられた胸腺および雄生殖器官の重量減少、胸腺の退縮、前立腺の低形成は体重減少等に基づく二次的な変化と考えられる。

血清生化学検査で500mg/kg 投与群でGOTおよびGPT 活性の上昇が、またBSP 排泄量の低下より肝臓に対する影響が示唆されたが、組織学的検査でKupffer細胞に軽度なヘモジデリン沈着が認められたのみで、GOT、GPT、BSPの結果を裏付ける所見は得られなかった。しかし、Kupffer細胞へのヘモジデリン沈着については、死亡例でうっ血の強くみられた小葉中心帯にヘモジデリン沈着が特に強くみられていることより、一般状態の悪化が循環動態の変動、すなわち、うっ血をひきおこしたためによるものと考えられる。しかし、これらの肝臓における変化は極く軽度で休薬により回復する可逆的な変化であった。

また、剖検において500mg/kg 投与群の腎臓は退色腫大し、重量の増加が認められた。しかし組織学的検査では、極く軽度な尿細管上皮の変性・壊死、刷子縁の脱落、尿細管の拡張、円柱の出現を認めたのみで、休薬により回復する可逆的な変化であった。電子顕微鏡検査では、近位尿細管上皮のミトコンドリア数の増加、ミトコンドリアの変形(環状)、ミトコンドリアのクリステの変化(縦走、層板状)、肝細胞においてメガミトコンドリアが認められたが、その他のオルガネラに変化はなかった。またこれらの変化は休薬により認められなくなった。これら腎臓にみられるミトコンドリアの変化は他のオルガネラの異常を伴わないこと、可逆的であることおよび慢性毒性試験(イヌ12ヵ月)では認められないことから重篤な障害に進展する病的変化ではないと思われる。

また、メガミトコンドリアの出現は生理的条件

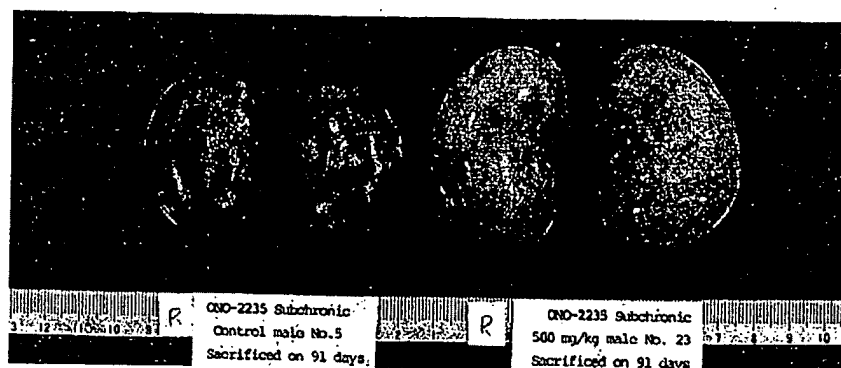


写真 1. 500mg/kg 投与群, 雄 (No.23)
腎臓: 退色腫大



写真 2. 500mg/kg 投与群, 雄 (No.19) ×200
腎臓: 尿細管上皮細胞の変性および壊死, 尿細管の拡張, 円柱の出現.

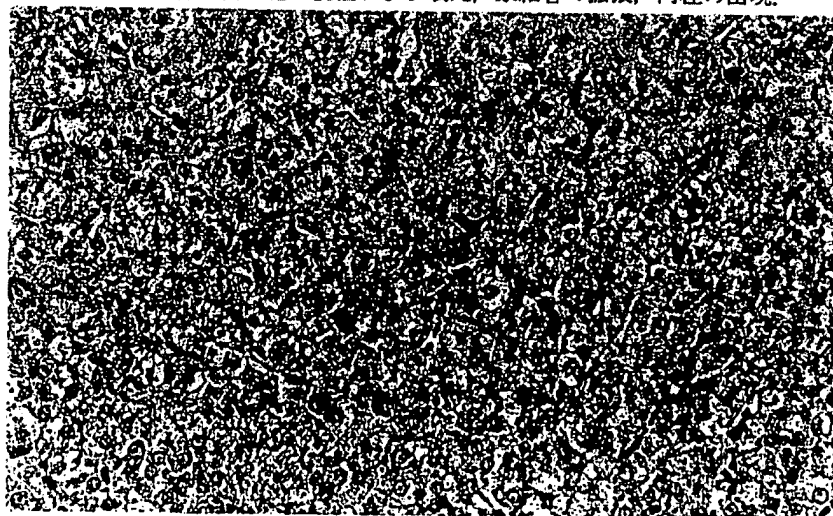


写真 3. 500mg/kg 投与群, 雄 (No.19) ×200
肝臓: kupffer 細胞に鉄反応陽性の物質を認める.

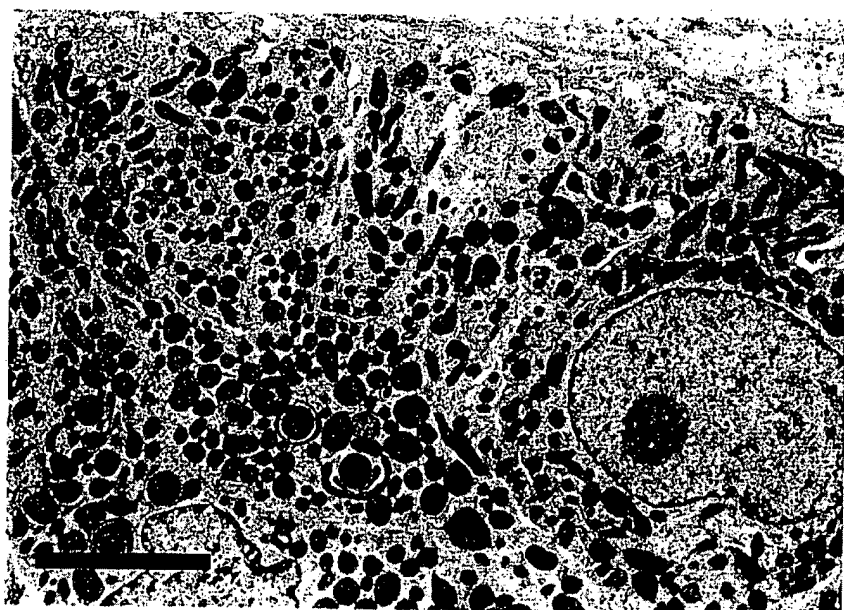


写真 4. 500mg/kg 投与群, 雄 (No19) スケール: 5 μ m
腎臓, 近位尿細管細胞: ミトコンドリア数の増加.

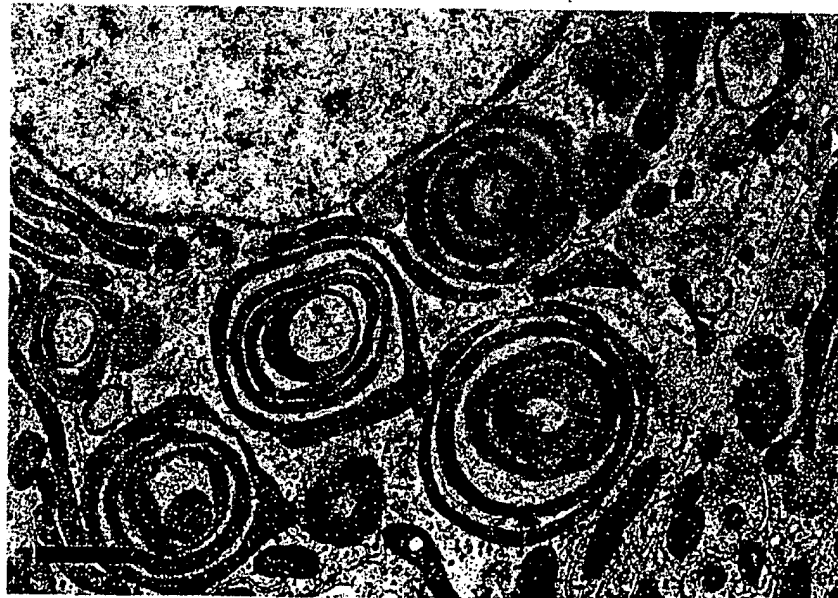


写真 5. 500mg/kg 投与群, 雄 (No22) スケール: 1 μ m
腎臓, 近位尿細管細胞: 環状あるいは同心円状のミトコン
ドリア, 一部に層板状のクリステを有する.

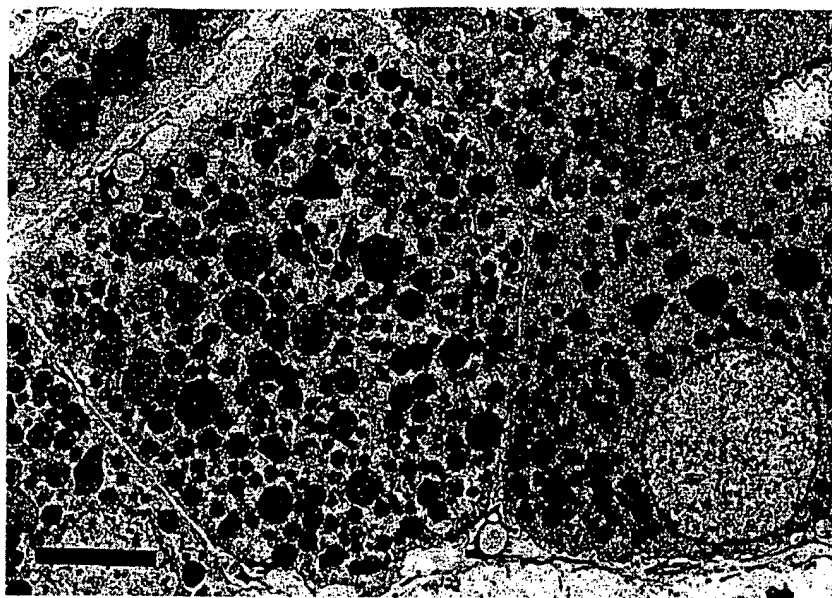


写真 6. 500mg/kg 投与群, 雄 (No19) スケール: 5 μ m
 肝臓: 肝細胞内にメガミトコンドリア, クッパー細胞内に
 ヘモジデリン様顆粒を認める.

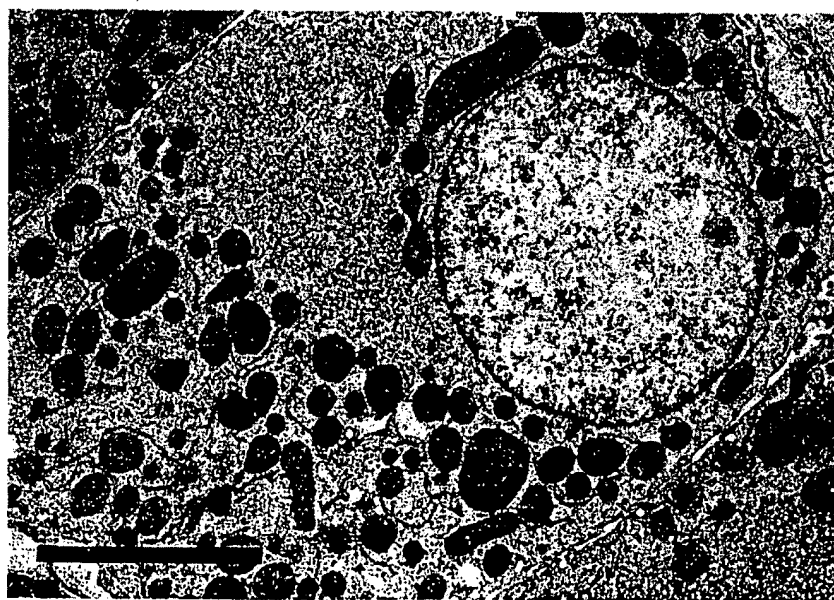


写真 7. 500mg/kg 投与群, 雌 (No519) スケール: 5 μ m
 肝臓: メガミトコンドリア内にラメラボディー物を認める.

表 1. Parameter, abbreviation and grade in urinalyses

Parameter	Abbreviation	-	±	+	++	+++	++++
Urinary volume (ml/16 HRS)							
Protein	PRO	Negative	50~20mg/dl	30mg/dl	100mg/dl	300mg/dl	2,000mg/dl More
Glucose	GLU	Negative	1/10% Glucose	1/4% Glucose	1/2% Glucose	1% Glucose	2% Glucose
Ketone body	KET	Negative		Slightly	Moderately	Strongly	
Occult blood	O.BL	Negative	Very slightly	Moderately	Strongly		
Bilirubin	BIL	Negative		Slightly	Moderately	Strongly	
Urobilinogen	UROB	(Ehrlich Unit/dl)					

表 2-1. Urinalyses in male dogs at the 2nd week before administration of ONO-2235

Group	No. of Animals	Volume (ml)	SG	pH					PRO					GLU					KET					
				5	6	7	8	9	-	±	+	++	+++	++++	-	±	+	++	+++	++++	-	+	++	+++
Control	6	218.0	1.029	0	3	1	1	1	3	0	2	1	0	0	6	0	0	0	0	0	6	0	0	0
Mean		108.3	0.014																					
S.D.																								
20.000	6	235.2	1.024	0	1	2	3	0	1	4	1	0	0	0	6	0	0	0	0	0	6	0	0	0
(mg/kg)		95.6	0.009																					
Mean																								
S.D.																								
100.000	6	252.7	1.028	0	1	0	5	0	2	3	1	0	0	0	6	0	0	0	0	0	6	0	0	0
(mg/kg)		89.3	0.010																					
Mean																								
S.D.																								
500.000	6	253.2	1.026	0	0	0	6	0	2	3	1	0	0	0	6	0	0	0	0	0	6	0	0	0
(mg/kg)		115.7	0.008																					
Mean																								
S.D.																								

表 2-2. Urinalyses in male dogs at the 2nd week before administration of ONO-2235

Group	No. of Animals	O.B.L.			BIL			UROB		
		-	±	+	-	+	++	0.1	1	2
Control	6	6	0	0	6	0	0	4	2	0
20.000 (mg/kg)	6	6	0	0	6	0	0	6	0	0
100.000 (mg/kg)	6	6	0	0	6	0	0	5	1	0
500.000 (mg/kg)	6	6	0	0	6	0	0	6	0	0

表 3-1. Urinalyses in male dogs at the 4th week after oral administration of ONO-2235

Group	No. of Animals	Volume (ml)	SG	pH					PRO					GLU					KET				
				5	6	7	8	9	-	±	+	++	+++	++++	-	±	+	++	+++	++++	-	+	++
Control	6	195.8	1.036	0	0	0	5	1	1	3	2	0	0	0	6	0	0	0	0	6	0	0	0
Mean		129.8	0.004																				
S.D.																							
20.000 (mg/kg)	6	231.2	1.030	0	0	1	3	2	1	2	3	0	0	0	6	0	0	0	0	6	0	0	0
Mean		141.4	0.010																				
S.D.																							
100.000 (mg/kg)	6	170.0	1.035	0	0	1	3	2	0	4	1	1	0	0	3	3	0	0	0	3	3	0	0
Mean		45.7	0.008																				
S.D.																							
500.000 (mg/kg)	6	356.8	1.028	0	0	0	5	1	4	0	2	0	0	0	3	3	0	0	0	3	3	0	0
Mean		115.2	0.010																				
S.D.																							

表 3-2. Urinalyses in male dogs at the 4th week after oral administration of ONO-2235

Effect of various doses of the drug on the rate of oral administration of UNO-2235																
Group	No. of Animals	OBL					BIL			UROB						
		-	±	+	++	+++	-	+	++	+++	0.1	1	2	4	8	12
Control	6	6	0	0	0	0	6	0	0	0	5	1	0	0	0	0
20.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	5	1	0	0	0	0
100.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	5	1	0	0	0	0
500.000 (mg/kg)	6	4	0	1	0	1	4	2	0	0	4	2	0	0	0	0

表 4-1. Urinalyses in male dogs at the 12th week after oral administration of ONO-2235

Group	No. of Animals	Volume (ml)	SG	pH			5	6	7	8	9	PRO			GLU			KET		
Control	6	235.8	1.032	0	1	1	3	1	1	2	3	0	0	0	0	0	0	0	0	0
Mean		95.6	0.012																	
S.D.																				
20.000 (mg/kg)	6	221.7	1.030	0	1	2	3	0	2	3	1	0	0	0	0	0	0	0	0	0
Mean		93.0	0.007																	
S.D.																				
100.000 (mg/kg)	6	172.2	1.032	0	1	0	4	1	0	4	2	0	0	0	0	0	0	0	0	0
Mean		60.6	0.007																	
S.D.																				
500.000 (mg/kg)	5	343.0	1.029	0	0	2	1	2	0	3	2	0	0	0	0	0	0	0	0	0
Mean		138.4	0.012																	
S.D.																				

表 4-2. Urinalyses in male dogs at the 12th week after oral administration of ONO-2235

Group	No. of Animals	O.BL			BIL			UROB			GLU			KET		
Control	6	6	0	0	0	6	0	0	0	4	2	0	0	0	0	0
20.000 (mg/kg)	6	6	0	0	0	6	0	0	0	5	1	0	0	0	0	0
100.000 (mg/kg)	6	5	1	0	0	6	0	0	0	5	1	0	0	0	0	0
500.000 (mg/kg)	5	4	1	0	0	5	0	0	0	3	2	0	0	0	0	0

表 5-1. Urinalyses in male dogs at the 4th week after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of Animals	Volume (ml)	SG	pH				PRO				GLU				KET			
				5	6	7	8	9	-	±	+	++	+++	++++	-	±	+	++	+++
Control	2	156.5	1.046	0	1	0	1	0	0	0	2	0	0	0	2	0	0	0	0
Mean		34.6	0.004																
S.D.																			
20.000 (mg/kg)	2	158.5	1.046	0	1	0	0	1	0	2	0	0	0	0	2	0	0	0	0
Mean		21.9	0.006																
S.D.																			
100.000 (mg/kg)	2	171.5	1.039	0	1	0	1	0	0	1	1	0	0	0	2	0	0	0	0
Mean		71.4	0.014																
S.D.																			
500.000 (mg/kg)	2	295.0	1.028	0	0	0	1	1	0	2	0	0	0	0	2	0	0	0	0
Mean		2.8	0.003																
S.D.																			

表 5-2. Urinalyses in male dogs at the 4th week after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of Animals	O.B.L			BIL			UROB											
		-	±	+	++	+++	-	+	++	+++	0.1	1	2	4	8	12			
Control	2	2	0	0	0	0	2	0	0	0	2	0	0	0	0	0			
20.000 (mg/kg)	2	2	0	0	0	0	2	0	0	0	2	0	0	0	0	0			
100.000 (mg/kg)	2	2	0	0	0	0	2	0	0	0	2	0	0	0	0	0			
500.000 (mg/kg)	2	2	0	0	0	0	2	0	0	0	2	0	0	0	0	0			

表 6-1. Urinalyses in female dogs at the 2nd week before administration of ONO-2235

Group	No. of Animals	Volume (ml)	SG	5	6	7	8	9	PRO			GLU			KET		
									-	±	+	±	+	+	+	-	
Control	6	191.7 99.7	1.027 0.005	0	0	1	4	1	2	4	0	0	0	0	0	6	
Mean																0	
S.D.																0	
20.000 (mg/kg)	6	209.2 95.0	1.029 0.007	0	0	5	1	0	4	0	2	0	0	0	0	6	
Mean																0	
S.D.																0	
100.000 (mg/kg)	6	211.7 87.8	1.035 0.006	0	1	1	2	2	2	2	2	0	0	0	0	6	
Mean																0	
S.D.			*													0	
500.000 (mg/kg)	6	289.2 137.7	1.032 0.007	0	1	0	4	1	0	3	3	0	0	0	0	6	
Mean																0	
S.D.																0	

Significantly different from control : * ($p < 0.05$)

表 6-2. Urinalyses in female dogs at the 2nd week before administration of ONO-2235

Group	No. of Animals	OBL			BIL			UROB								
		-	±	+	++	+++	-	+	++	+++	0.1	1	2	4	8	12
Control	6	6	0	0	0	0	6	0	0	0	5	1	0	0	0	0
20.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	5	1	0	0	0	0
100.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	6	0	0	0	0	0
500.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	6	0	0	0	0	0

表 7-1. Urinalyses in female dogs at the 4th week after oral administration of ONO-2235

Group	No. of Animals	Volume (ml)	SG	pH				PRO				GLU				KET			
Control	6	249.0	1.030	0	0	0	5	1	3	2	0	1	0	0	0	6	0	0	0
Mean		125.6	0.013																
S.D.																			
20.000 (mg/kg)	6	246.7	1.027	0	0	2	3	1	3	2	1	0	0	0	0	6	0	0	0
Mean		64.9	0.008																
S.D.																			
100.000 (mg/kg)	6	251.3	1.036	0	0	1	3	2	0	1	5	0	0	0	0	5	1	0	0
Mean		157.6	0.007																
S.D.																			
500.000 (mg/kg)	6	327.5	1.029	0	0	4	2	0	3	3	0	0	0	0	0	3	3	0	0
Mean		130.1	0.011																
S.D.																			

表 7-2. Urinalyses in female dogs at the 4th week after oral administration of ONO-2235

Group	No. of Animals	O.BL			BIL			UROB		
		-	±	+	-	+	+	0.1	1	2
Control	6	6	0	0	6	0	0	4	2	0
20.000 (mg/kg)	6	6	0	0	6	0	0	5	1	0
100.000 (mg/kg)	6	6	0	0	6	0	0	3	3	0
500.000 (mg/kg)	6	4	2	0	5	1	0	3	3	0

表 8-1. Urinalyses in female dogs at the 12th week after oral administration of ONO-2235

Group	No. of Animals	Volume (ml)	SG	5	6	7	8	9	-	±	+	++	+++	++++	-	±	+	++	+++	++++	-	+	++	+++
Control	6	193.3	1.024	0	0	2	2	2	3	1	2	0	0	0	6	0	0	0	0	0	0	6	0	0
Mean		68.4	0.006																					
S.D.																								
20.000 (mg/kg)	6	236.7	1.028	0	2	0	4	0	3	1	2	0	0	0	6	0	0	0	0	0	0	6	0	0
Mean		156.7	0.009																					
S.D.																								
100.000 (mg/kg)	6	169.2	1.034	0	0	0	2	4	1	3	1	1	0	0	6	0	0	0	0	0	0	6	0	0
Mean		71.0	0.007																					
S.D.																								
500.000 (mg/kg)	6	317.5	1.027	0	1	1	3	1	2	2	2	0	0	0	6	0	0	0	0	0	0	2	4	0
Mean		97.2	0.007																					
S.D.																								

Significantly different from control : * (p<0.05)

表 8-2. Urinalyses in female dogs at the 12th week after oral administration of ONO-2235

Group	No. of Animals	O.B.L.	-	±	+	++	+++	-	+	++	+++	0.1	1	2	4	8	12
Control	6	6	0	0	0	0	0	6	0	0	0	6	0	0	0	0	0
20.000 (mg/kg)	6	5	1	0	0	0	0	6	0	0	0	6	0	0	0	0	0
100.000 (mg/kg)	6	5	0	0	1	0	0	6	0	0	0	4	2	0	0	0	0
500.000 (mg/kg)	6	3	2	1	0	0	0	6	0	0	0	5	1	0	0	0	0

表 9-1. Urinalyses in female dogs at the 4th week after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of Animals	Volume (ml)	SC	pH				PRO				GLU				KET			
				5	6	7	8	9	-	±	+	++	+++	++++	-	±	+	++	+++
Control	2	185.5	1.039	0	1	0	1	0	1	0	1	0	0	0	2	0	0	0	0
Mean		44.5	0.015																
S.D.																			
20.000 (mg/kg)	2	188.5	1.034	0	0	1	1	0	0	1	1	0	0	0	2	0	0	0	0
Mean		24.7	0.013																
S.D.																			
100.000 (mg/kg)	2	266.0	1.036	0	0	1	1	0	1	1	0	0	0	0	2	0	0	0	0
Mean		149.9	0.011																
S.D.																			
500.000 (mg/kg)	2	295.5	1.040	0	0	0	1	1	0	0	2	0	0	0	2	0	0	0	0
Mean		197.3	0.007																
S.D.																			

表 9-2. Urinalyses in female dogs at the 4th week after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of Animals	O.B.L				BIL				UROB											
		-	±	+	++	+++	-	+	++	+++	0.1	1	2	4	8	12					
Control	2	2	0	0	0	0	2	0	0	0	1	1	0	0	0	0					
20.000 (mg/kg)	2	1	0	0	0	1	2	0	0	0	2	0	0	0	0	0					
100.000 (mg/kg)	2	2	0	0	0	0	2	0	0	0	2	0	0	0	0	0					
500.000 (mg/kg)	2	1	0	1	0	0	2	0	0	0	2	0	0	0	0	0					

表 10. Urinary electrolytes in male dogs at the 2nd week before administration of ONO-2235

Group	No. of animals	Ca ²⁺ mEq/l	Na ⁺ mEq/l	K ⁺ mEq/l	Cl ⁻ mEq/l
Control					
Mean	6	0.95	105.4	184.50	113.17
S.D.		0.52	40.8	55.60	50.69
20.000 (mg/kg)					
Mean	6	0.80	106.2	184.00	107.33
S.D.		0.20	27.6	64.21	44.10
100.000 (mg/kg)					
Mean	6	0.69	89.3	186.67	106.00
S.D.		0.42	57.9	66.75	42.43
500.000 (mg/kg)					
Mean	6	0.97	81.7	195.00	91.17
S.D.		0.38	26.8	67.68	34.31

表 11. Urinary electrolytes in male dogs at the 4th week after administration of ONO-2235

Group	No. of animals	Ca ²⁺ mEq/l	Na ⁺ mEq/l	K ⁺ mEq/l	Cl ⁻ mEq/l
Control					
Mean	6	0.33	77.6	175.50	109.67
S.D.		0.19	53.0	79.20	61.20
20.000 (mg/kg)					
Mean	6	0.43	76.9	161.17	81.50
S.D.		0.31	31.6	73.51	57.68
100.000 (mg/kg)					
Mean	6	0.58	71.7	202.67	130.33
S.D.		0.28	64.7	58.67	44.03
500.000 (mg/kg)					
Mean	6	0.79	33.5	141.00	49.67
S.D.		0.35	12.9	95.62	22.13

Significantly different from control : * (p<0.05)

表 12. Urinary electrolytes in male dogs at the 12th week after oral administration of ONO-2235

Group	No. of animals	Ca ²⁺ mEq/l	Na ⁺ mEq/l	K ⁺ mEq/l	Cl ⁻ mEq/l
Control					
Mean	6	0.57	90.6	161.67	129.50
S.D.		0.83	49.2	41.23	42.59
20.000 (mg/kg)					
Mean	6	0.63	95.7	188.00	134.67
S.D.		0.80	34.8	69.91	62.68
100.000 (mg/kg)					
Mean	6	0.50	83.6	168.83	164.33
S.D.		0.49	33.5	89.60	59.96
500.000 (mg/kg)					
Mean	5	0.43	49.3	108.00	70.00
S.D.		0.84	40.5	69.22	47.02

表 13. Urinary electrolytes in male dogs at the 4th week after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	Ca ²⁺ mEq/l	Na ⁺ mEq/l	K ⁺ mEq/l	Cl ⁻ mEq/l
Control					
Mean	2	0.82	118.0	181.50	130.00
S.D.		0.42	5.3	4.95	7.07
20.000 (mg/kg)					
Mean	2	0.92	88.3	160.00	112.50
S.D.		0.94	46.2	25.46	19.09
100.000 (mg/kg)					
Mean	2	0.98	98.5	198.50	135.00
S.D.		0.78	39.6	26.16	14.14
500.000 (mg/kg)					
Mean	2	1.45	32.6	54.50	44.00
S.D.		0.23	19.8	38.89	14.14

表 14. Urinary electrolytes in female dogs at the 2nd week before administration of ONO-2235

Group	No. of animals	Ca ²⁺ mEq/l	Na ⁺ mEq/l	K ⁺ mEq/l	Cl ⁻ mEq/l
Control					
Mean	6	1.19	60.8	247.00	103.33
S.D.		0.30	24.4	84.57	64.48
20.000 (mg/kg)					
Mean	6	1.12	74.6	183.33	105.50
S.D.		0.37	42.0	62.94	43.69
100.000 (mg/kg)					
Mean	6	1.17	78.4	248.67	139.50
S.D.		0.46	28.4	65.28	59.01
500.000 (mg/kg)					
Mean	6	1.10	68.0	181.50	82.50
S.D.		0.32	36.5	80.57	31.85

表 15. Urinary electrolytes in female dogs at the 4th week after oral administration of ONO-2235

Group	No. of animals	Ca ²⁺ mEq/l	Na ⁺ mEq/l	K ⁺ mEq/l	Cl ⁻ mEq/l
Control					
Mean	6	0.45	48.7	188.67	94.17
S.D.		0.16	18.2	68.91	48.47
20.000 (mg/kg)					
Mean	6	0.70	37.8	138.67	85.50
S.D.		0.27	24.3	61.47	40.55
100.000 (mg/kg)					
Mean	6	0.66	73.5	211.17	119.67
S.D.		0.32	36.8	79.93	51.77
500.000 (mg/kg)					
Mean	6	0.66	36.5	104.67	57.17
S.D.		0.37	19.6	48.41	27.04

Significantly different from control : * (p<0.05)

表 16. Urinary electrolytes in female dogs at the 12th week after oral administration of ONO-2235

Group	No. of animals	Ca ²⁺ mEq/l	Na ⁺ mEq/l	K ⁺ mEq/l	Cl ⁻ mEq/l
Control					
Mean	6	0.33	62.5	162.17	138.50
S.D.		0.73	28.6	70.54	72.15
20.000 (mg/kg)					
Mean	6	0.51	73.2	170.50	134.50
S.D.		1.16	40.3	86.66	82.49
100.000 (mg/kg)					
Mean	6	0.25	78.3	173.00	90.83
S.D.		0.41	41.5	100.27	65.65
500.000 (mg/kg)					
Mean	6	0.56	77.9	188.17	133.00
S.D.		1.28	36.0	86.24	54.84

表 17. Urinary electrolytes in female dogs at the 4th week after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	Ca ²⁺ mEq/l	Na ⁺ mEq/l	K ⁺ mEq/l	Cl ⁻ mEq/l
Control					
Mean	2	0.65	119.7	226.00	133.50
S.D.		0.54	20.8	49.50	53.03
20.000 (mg/kg)					
Mean	2	1.17	90.7	180.50	91.00
S.D.		0.17	0.2	10.61	5.66
100.000 (mg/kg)					
Mean	2	0.68	73.7	163.50	99.00
S.D.		0.42	13.8	41.72	28.28
500.000 (mg/kg)					
Mean	2	0.78	118.5	187.50	86.50
S.D.		0.10	100.2	102.53	74.25

表 18. Parameter and abbreviation in urinary sediments

Parameter	Abbreviation	Grade
Epithelial cell	EPC	— Negative
Lecucocytes	LC	± Very slightly
Erythrocytes	EC	+ Slightly
Bacteria	BAC	++ Moderately
Casts	CAS	+++ Markedly
Ammoniaco-magnesian phosphates	AMP	
Oxalate crystals	CO	
Protozoa	PROT	
Fat globule	FG	
Mycete	MYC	
Sperma	SPM (male only)	
Oocyst	OOC	
Insect	INS	
Mucus filament	MF	
Others	OT	

表 19-1. Urinary sediments in male dogs at the 2nd week before administration of ONO-2235

Group	No. of animals	EPC			LC			EC			BAC			CAS		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	0	0	2	0	4	4	1	0	1	0	6	0	0	0	0
20.000 (mg/kg)	6	0	0	1	1	4	2	3	0	1	0	6	0	0	0	0
100.000 (mg/kg)	6	0	0	1	1	4	2	1	2	1	0	6	0	0	0	0
500.000 (mg/kg)	6	0	0	0	1	5	0	4	1	1	0	6	0	0	0	0

表 19-2. Urinary sediments in male dogs at the 2nd week before administration of ONO-2235

Group	No. of animals	AMP			CO			PROT			FG			MYC		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	3	0	0	0	3	6	0	0	0	0	6	0	0	0	0
20.000 (mg/kg)	6	5	0	0	0	1	6	0	0	0	0	6	0	0	0	0
100.000 (mg/kg)	6	1	0	0	0	5	6	0	0	0	0	6	0	0	0	0
500.000 (mg/kg)	6	1	0	0	0	5	6	0	0	0	0	6	0	0	0	0

表 19-3. Urinary sediments in male dogs at the 2nd week before administration of ONO-2235

Group	No. of animals	SPM			OOC			INS			MF			OT		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	1
20.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0
100.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0
500.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	2

表 20-1. Urinary sediments in male dogs at the 4th week after oral administration of ONO-2235

Group	No. of animals	EPC			LC			EC			BAC			CAS		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	0	0	1	2	3	5	1	0	0	0	5	1	0	0	0
20.000 (mg/kg)	6	0	0	1	0	5	5	0	0	1	0	4	2	0	0	0
100.000 (mg/kg)	6	0	0	0	0	6	6	0	0	0	0	5	1	0	0	0
500.000 (mg/kg)	5	0	0	0	0	6	6	0	0	0	0	4	1	1	0	0

表 20-2. Urinary sediments in male dogs at the 4th week after oral administration of ONO-2235

Group	No. of animals	AMP			CO			PROT			FG			MYC		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	0	0	0	1	5	6	0	0	0	0	6	0	0	0	0
20.000 (mg/kg)	6	2	0	0	0	4	6	0	0	0	0	6	0	0	0	0
100.000 (mg/kg)	6	1	0	0	0	5	6	0	0	0	0	6	0	0	0	0
500.000 (mg/kg)	5	3	0	0	0	3	6	0	0	0	0	6	0	0	0	0

表 20-3. Urinary sediments in male dogs at the 4th week after oral administration of ONO-2235

Group	No. of animals	SPM			OOC			INS			MF			OT		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	5	0	1	0	0	6	0	0	0	0	6	0	0	0	1
20.000 (mg/kg)	6	5	0	1	0	0	6	0	0	0	0	6	0	0	0	2
100.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	2
500.000 (mg/kg)	6	4	0	2	0	0	6	0	0	0	0	6	0	0	0	0

表 21-1. Urinary sediments in male dogs at the 12th week after oral administration of ONO-2235

Group	No. of animals	EPC			LC			EC			BAC			CAS		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	0	0	0	2	4	2	1	0	1	2	5	1	0	0	0
20.000 (mg/kg)	6	0	0	0	1	5	2	2	0	1	1	6	0	0	0	0
100.000 (mg/kg)	6	0	0	0	0	6	4	0	0	0	2	5	1	0	0	0
500.000 (mg/kg)	5	0	0	0	0	5	3	1	0	1	0	4	1	0	0	0

表 21-2. Urinary sediments in male dogs at the 12th week after oral administration of ONO-2235

Group	No. of animals	AMP			CO			PROT			FG			MYC		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	2	0	0	0	4	6	0	0	0	0	6	0	0	0	0
20.000 (mg/kg)	6	1	0	0	0	5	6	0	0	0	0	6	0	0	0	0
100.000 (mg/kg)	6	0	0	0	1	5	6	0	0	0	0	6	0	0	0	0
500.000 (mg/kg)	5	2	0	0	0	3	5	0	0	0	0	5	0	0	0	0

表 21-3. Urinary sediments in male dogs at the 12th week after oral administration of ONO-2235

Group	No. of animals	SPM			OOC			INS			MF			OT		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	1	0	5	0	0	6	0	0	0	0	6	0	0	0	0
20.000 (mg/kg)	6	2	0	4	0	0	6	0	0	0	0	6	0	0	0	0
100.000 (mg/kg)	6	3	0	3	0	0	6	0	0	0	0	6	0	0	0	0
500.000 (mg/kg)	5	4	0	1	0	0	5	0	0	0	0	5	0	0	0	0

表 22-1. Urinary sediments in male dogs at the 4th week after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	EPC			LC			EC			BAC			CAS		
		-	±	+	-	±	+	-	±	+	-	±	+	-	±	+
Control	2	0	0	1	0	0	1	2	0	0	0	0	0	2	0	0
20.000 (mg/kg)	2	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0
100.000 (mg/kg)	2	0	0	1	0	0	0	2	0	0	0	0	0	2	0	0
500.000 (mg/kg)	2	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0

表 22-2. Urinary sediments in male dogs at the 4th week after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	AMP			CO			PROT			FG			MYC		
		-	±	+	-	±	+	-	±	+	-	±	+	-	±	+
Control	2	0	0	0	2	1	0	0	1	2	0	0	0	2	0	0
20.000 (mg/kg)	2	0	0	0	0	2	0	0	0	2	0	0	0	2	0	0
100.000 (mg/kg)	2	0	0	0	0	2	0	0	0	2	0	0	0	2	0	0
500.000 (mg/kg)	2	0	0	0	0	2	0	0	0	2	0	0	0	2	0	0

表 22-3. Urinary sediments in male dogs at the 4th week after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	SPM			OOC			INS			MF			OT		
		-	±	+	-	±	+	-	±	+	-	±	+	-	±	+
Control	2	1	0	1	0	0	0	2	0	0	0	0	0	2	0	0
20.000 (mg/kg)	2	1	0	1	0	0	0	2	0	0	0	0	0	2	0	0
100.000 (mg/kg)	2	1	0	1	0	0	0	2	0	0	0	0	0	2	0	0
500.000 (mg/kg)	2	0	0	2	0	0	0	2	0	0	0	0	0	2	0	0

表 23-1. Urinary sediments in female dogs at the 2nd week before administration of ONO-2235

Group	No. of animals	EPC			LC			EC			BAC			CAS		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	0	1	2	0	3	4	2	0	0	0	6	0	0	0	0
20.000 (mg/kg)	6	0	1	2	0	3	3	2	0	1	0	6	0	0	0	0
100.000 (mg/kg)	6	0	0	0	0	6	5	0	0	1	0	6	0	0	0	0
500.000 (mg/kg)	6	0	0	0	1	5	2	1	2	1	0	6	0	0	0	0

表 23-2. Urinary sediments in female dogs at the 2nd week before administration of ONO-2235

Group	No. of animals	AMP			CO			PROT			FG			MYC		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	1	0	0	0	5	6	0	0	0	0	6	0	0	0	0
20.000 (mg/kg)	6	2	0	0	0	4	6	0	0	0	0	6	0	0	0	0
100.000 (mg/kg)	6	0	0	0	0	6	6	0	0	0	0	6	0	0	0	0
500.000 (mg/kg)	6	0	0	0	0	6	6	0	0	0	0	6	0	0	0	0

表 23-3. Urinary sediments in female dogs at the 2nd week before administration of ONO-2235

Group	No. of animals	OOC			INS			MF			OT					
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0
20.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	2
100.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	1
500.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	1

表 24-1. Urinary sediments in female dogs at the 4th week after oral administration of ONO-2235

Group	No. of animals	EPC			LC			EC			BAC			CAS		
		-	±	+	-	±	+	-	±	+	-	±	+	-	±	+
Control	6	0	0	2	0	4	5	1	0	0	6	0	0	0	3	2
20.000 (mg/kg)	6	0	1	2	1	2	4	1	0	1	5	1	0	0	5	1
100.000 (mg/kg)	6	0	0	0	0	6	5	1	0	0	5	1	0	0	5	1
500.000 (mg/kg)	6	0	0	0	0	6	6	0	0	0	3	3	0	0	5	1

表 24-2. Urinary sediments in female dogs at the 4th week after oral administration of ONO-2235

Group	No. of animals	AMP			CO			PROT			FG			MYC		
		-	±	+	-	±	+	-	±	+	-	±	+	-	±	+
Control	6	2	0	0	4	6	0	0	0	0	6	0	0	0	6	0
20.000 (mg/kg)	6	3	0	0	3	5	0	0	0	1	6	0	0	0	5	1
100.000 (mg/kg)	6	3	0	0	3	6	0	0	0	0	6	0	0	0	6	0
500.000 (mg/kg)	6	1	0	0	5	6	0	0	0	0	6	0	0	0	6	0

表 24-3. Urinary sediments in female dogs at the 4th week after oral administration of ONO-2235

Group	No. of animals	OOC			INS			MF			OT		
		-	±	+	-	±	+	-	±	+	-	±	+
Control	6	6	0	0	6	0	0	6	0	0	6	0	0
20.000 (mg/kg)	6	6	0	0	6	0	0	6	0	0	5	0	0
100.000 (mg/kg)	6	6	0	0	6	0	0	6	0	0	4	0	0
500.000 (mg/kg)	6	6	0	0	6	0	0	6	0	0	5	0	0

表 25-1. Urinary sediments in female dogs at the 12th week after oral administration of ONO-2235

Group	No. of animals	EPC			LC			EC			BAC			CAS		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	0	0	1	3	2	2	1	0	3	0	5	1	0	0	0
20.000 (mg/kg)	6	0	1	1	0	4	2	2	2	0	0	5	1	0	0	0
100.000 (mg/kg)	6	0	0	0	0	6	2	2	1	1	0	6	0	0	0	0
500.000 (mg/kg)	6	0	0	0	0	6	4	1	0	1	0	6	0	0	0	0

表 25-2. Urinary sediments in female dogs at the 12th week after oral administration of ONO-2235

Group	No. of animals	AMP			CO			PROT			FG			MYC		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	3	0	0	0	3	6	0	0	0	0	6	0	0	0	0
20.000 (mg/kg)	6	2	0	0	0	4	6	0	0	0	0	6	0	0	0	0
100.000 (mg/kg)	6	0	0	0	0	6	6	0	0	0	0	6	0	0	0	0
500.000 (mg/kg)	6	2	0	0	0	4	6	0	0	0	0	6	0	0	0	0

表 25-3. Urinary sediments in female dogs at the 12th week after oral administration of ONO-2235

Group	No. of animals	OOC			INS			MF			OT					
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0
20.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0
100.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0
500.000 (mg/kg)	6	6	0	0	0	0	6	0	0	0	0	6	0	0	0	0

表 26-1. Urinary sediments in female dogs at the 4th week after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	EPC			LC			EC			BAC			CAS		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	2	0	0	1	1	0	2	0	0	0	0	2	0	0	0	0
20.000 (mg/kg)	2	0	0	0	0	2	2	0	0	0	0	1	1	0	0	0
100.000 (mg/kg)	2	0	0	0	0	2	2	0	0	0	0	2	0	0	0	0
500.000 (mg/kg)	2	0	0	0	0	2	2	0	0	0	0	2	0	0	0	0

表 26-2. Urinary sediments in female dogs at the 4th week after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	AMP			CO			PROT			FG			MYC		
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	2	1	0	0	0	1	2	0	0	0	0	2	0	0	0	0
20.000 (mg/kg)	2	0	0	0	0	2	1	0	0	0	1	2	0	0	0	0
100.000 (mg/kg)	2	0	0	0	0	2	2	0	0	0	0	2	0	0	0	0
500.000 (mg/kg)	2	0	0	0	0	2	2	0	0	0	0	2	0	0	0	0

表 26-3. Urinary sediments in female dogs at the 4th week after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	OOC			INS			MF			OT					
		-	±	+	++	+++	-	±	+	++	+++	-	±	+	++	+++
Control	2	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0
20.000 (mg/kg)	2	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0
100.000 (mg/kg)	2	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0
500.000 (mg/kg)	2	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0

表 27-1. Hematological findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	RBC ($10^3/\text{mm}^3$)				Ht (%)				
	Before ^{a)}	30 day	90 day	Recovery ^{b)}	Before	30 day	90 day	Recovery	
Control	Mean S.D.	669.0±44.6	683.2±74.7	684.5±70.8	628.5±140.7	44.3±2.1	46.0±4.3	47.0±4.7	43.0±8.5
20	Mean S.D.	600.0±30.7	583.3±31.7	625.0±44.7	652.5±30.4	40.0±2.4	41.2±2.1	41.2±2.4	44.7±3.9
100	Mean S.D.	625.0±38.2	613.0±62.0	624.3±29.1	621.5±115.3	43.7±2.7	42.1±3.3	43.1±3.2	39.5±3.5
500	Mean S.D.	603.5±53.6	631.3±37.6	643.2±61.6	584.5±2.1	41.0±3.5	43.9±2.3	43.6±3.8	42.0±0.0

a) : At the 5th day before starting

b) : At the 30th day of withdrawal

Significantly different from control : *

(p<0.05)

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 27-2. Hematological findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	Hb (g/dl)			Recovery ^{b)}	Plat (10 ³ /mm ³)				
	Before ^{a)}	30 day	90 day		Before	30 day	90 day		
Control	Mean S.D.	14.7±1.1	15.2±1.4	15.2±1.6*	14.2±2.7	30.1±9.3	28.1±6.8	29.3±3.6	28.3±3.7
20	Mean S.D.	13.3±0.8	13.6±0.6	14.0±0.9	15.4±0.8	30.1±5.3	25.6±4.5	28.3±4.1	23.5±2.5
100	Mean S.D.	14.1±0.9	14.0±1.5	14.2±1.0	14.2±1.8	24.7±4.6	27.9±4.0	51.8±38.5	27.5±1.2
500	Mean S.D.	13.8±1.3	14.5±0.9	14.9±1.6	14.1±0.7	28.4±4.8	29.8±9.9	37.9±4.7*	28.6±7.8

a) : At the 5th day before starting

b) : At the 30th day of withdrawal

Significantly different from control : * (p<0.05)

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 27-3. Hematological findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	WBC ($10^3/\text{mm}^3$)				Lym (%)				
	Before ^{a)}	30 day	90 day	Recovery ^{b)}	Before	30 day	90 day	Recovery	
Control	Mean S.D.	118.7±21.0	104.2±40.5	101.5±35.9	116.5±14.8	39.3±5.1	27.2±7.6	28.5±7.3	35.0±4.2
20	Mean S.D.	125.0±23.0	90.8±14.1	87.0±12.4	91.0±11.3	30.5±8.8	36.0±6.0	27.7±7.8	30.0±2.8
100	Mean S.D.	* 91.0±10.3	76.7±10.6	101.0±24.6	123.0±32.5	35.8±5.9	* 40.7±9.6	28.3±8.0	28.5±10.6
500	Mean S.D.	110.0±34.9	77.2±21.6	73.8±13.0	102.0±26.9	30.3±8.9	33.0±13.1	23.0±4.7	36.0±14.1

a) : At the 5th day before starting b) : At the 30th day of withdrawal
 Significantly different from control : * (p<0.05)

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 27-4. Hematological findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		Neut-st (%)		Recovery ^{b)}	Neut-seg (%)		Recovery
		Before ^{a)}	30 day	90 day	Before	30 day	90 day
Control	Mean S.D.	3.0±2.2	2.2±2.2	5.0±2.2	54.8±6.1	65.0±8.7	63.2±8.7
20	Mean S.D.	4.0±2.8	3.3±1.6	4.0±1.7	62.2±11.1	55.3±9.2	64.7±8.3
100	Mean S.D.	2.7±1.2	1.5±1.0	3.0±1.3	55.3±7.9	54.8±9.0	64.2±5.3
500	Mean S.D.	1.8±2.2	3.3±2.4	4.4±2.6	62.5±8.2	61.2±12.4	69.4±2.3

a) : At the 5th day before starting b) : At the 30th day of withdrawal

表 27-5. Hematological findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		Mono (%)		Recovery ^{b)}	Eosin (%)		Recovery
		Before ^{a)}	30 day	90 day	Before	30 day	90 day
Control	Mean S.D.	1.0±0.9	1.0±0.0	0.8±1.0	1.8±1.7	4.7±3.4	2.5±2.7
20	Mean S.D.	0.7±0.8	0.3±0.5	0.8±0.8	3.0±3.3	5.0±3.9	2.8±2.5
100	Mean S.D.	0.7±0.8	0.3±0.5	0.3±0.8	5.3±3.4	2.7±1.2	4.2±3.0
500	Mean S.D.	1.0±0.9	0.8±0.4	0.6±0.9	4.3±2.3	1.7±1.5	2.6±3.1

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 27-6. Hematological findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		Baso (%)		Recovery ^{b)}	Ret (%)		Recovery
		Before ^{a)}	30 day	90 day	Before	30 day	90 day
Control	Mean S.D.	0.0±0.0	0.0±0.0	0.0±0.0	1.5±0.5	1.7±0.8	2.3±0.8
20	Mean S.D.	0.0±0.0	0.0±0.0	0.0±0.0	1.8±0.8	1.5±0.8	2.0±0.6
100	Mean S.D.	0.2±0.4	0.0±0.0	0.0±0.0	1.8±0.8	1.7±0.5	2.0±0.0
500	Mean S.D.	0.0±0.0	0.0±0.0	0.0±0.0	1.3±0.5	1.3±0.5	2.4±0.5

a) : At the 5th day before starting b) : At the 30th day of withdrawal

表 27-7. Hematological findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	PT (sec)		PTT (sec)			
	Before ^{a)}	30 day	90 day	Recovery ^{b)}		
Control	Mean S.D.	7.1±0.6	8.0±0.5	7.9±1.2	8.8±0.6	
20	Mean S.D.	7.1±0.7	7.6±0.7	7.8±0.6	8.8±0.1	
100	Mean S.D.	8.3±0.9	8.2±0.6	7.6±0.5	8.7±0.4	
500	Mean S.D.	7.8±0.6	8.4±0.7	7.2±0.5	8.4±0.1	
a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05), ** (p<0.01)						
	Before		30 day		90 day	
	Before		30 day		90 day	
Control	13.4±1.7		14.8±0.7		14.7±1.2	
20	13.4±0.6		12.5±1.0		16.1±1.2	
100	14.1±1.2		15.0±1.2		13.0±0.9	
500	15.5±0.8		14.9±2.1		13.2±1.9	

表 27-8. Hematological findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	MCV (μ^3)			MCH ($\gamma\gamma$)					
	Before ^{a)}	30 day	90 day	Recovery ^{b)}	Before	30 day	90 day	Recovery	
Control	Mean S.D.	66.4±2.6	67.4±2.0	68.7±1.6	68.6±1.9	21.9±0.7	22.2±0.5	22.3±0.9	22.7±0.8
20	Mean S.D.	66.7±1.7	70.7±4.7	66.1±3.2	68.5±2.8	22.3±0.7	23.5±1.8	22.4±0.5	23.7±0.1
100	Mean S.D.	69.9±3.8	68.9±3.9	69.0±2.9	64.1±6.2	22.6±1.0	22.9±0.9	22.8±1.0	23.0±1.3
500	Mean S.D.	68.1±4.3	69.6±3.2	67.9±4.3	71.9±6.3	22.9±1.1	23.0±0.5 [*]	23.1±0.4	24.1±1.3

表 27-9. Hematological findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		MCHC (%)			Recovery ^{b)}
		Before ^{a)}	30 day	90 day	
Control	Mean S.D.	33.1±1.3	33.0±1.0	32.4±1.7	33.0±0.3
20	Mean S.D.	33.4±0.9	33.2±0.6	34.0±1.5	34.6±1.3
100	Mean S.D.	32.3±0.7	33.3±1.8	33.1±0.9	35.9±1.4
500	Mean S.D.	33.7±1.7	33.1±1.3	34.2±1.8	33.6±1.7

表 28-1. Hematological findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		RBC ($10^3/\text{mm}^3$)			Recovery ^{b)}	Ht (%)			
		Before ^{a)}	30 day	90 day		Before	30 day	90 day	Recovery
Control	Mean S.D.	617.7±37.2	612.5±49.6	593.8±42.3	593.0±53.7	40.1±2.1	42.7±2.9	39.6±2.5	40.5±2.1
20	Mean S.D.	619.7±28.0	617.0±45.6	621.3±43.5	627.5±54.4	43.0±2.9	43.1±4.1	41.7±4.2	42.2±3.2
100	Mean S.D.	633.5±52.0	603.2±70.0	604.7±43.1	560.5±34.6	42.8±4.8	43.1±5.4	40.9±2.7	39.2±3.9
500	Mean S.D.	662.5±65.2	621.2±21.3	626.8±63.9	578.0±33.9	44.6±3.4 [*]	42.7±2.6	42.1±3.7	40.0±2.8

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 28-2. Hematological findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	Hb (g/dl)					Plat ($10^3/\text{mm}^3$)			
	Before ^{a)}	30 day	90 day	Recovery ^{b)}		Before	30 day	90 day	Recovery
Control	Mean	14.0±1.2	13.8±0.9	13.5±0.7	13.7±0.8	27.6±6.4	25.8±6.0	41.1±21.8	32.3±6.6
	S.D.								
20	Mean	14.3±1.2	14.5±1.2	13.8±1.2	14.5±0.8	25.5±5.3	27.9±4.4	27.2±6.2	28.5±1.1
	S.D.								
100	Mean	14.3±1.5	14.3±1.7	13.7±1.4	13.1±1.5	27.5±2.4	27.9±3.7	30.3±5.7	33.4±6.5
	S.D.								
500	Mean	15.1±1.6	15.2±0.8	14.4±1.5	13.1±0.4	26.3±3.5	28.3±3.0	32.5±9.1	40.4±8.4
	S.D.								

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 28-3. Hematological findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		WBC ($10^3/\text{mm}^3$)				Recovery ^{b)}	Lym (%)		
		Before ^{a)}	30 day	90 day	Before		30 day	90 day	Recovery
Control	Mean S.D.	109.3±40.1	90.0±18.2	108.2±27.6	112.0±0.0	41.0±11.1	37.3±9.0	28.8±7.6	36.5±0.7
20	Mean S.D.	115.3±26.3	99.7±18.9	106.5±21.4	110.5±3.5	31.3±9.6	28.8±7.1	24.0±3.9	27.5±3.5
100	Mean S.D.	124.5±23.5	95.0±20.9	106.2±21.8	124.5±13.4	28.0±10.2	34.8±10.9	27.8±4.7	22.0±12.7
500	Mean S.D.	112.0±20.1	96.0±19.2	99.3±12.7	126.5±3.5	32.5±8.4	38.0±11.7	30.2±7.9	24.0±1.4

a) : At the 5th day before starting b) : At the 30th day of withdrawal

表 28-4. Hematological findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		Neut-st (%)			Recovery ^{b)}	Neut-seg (%)		
		Before ^{a)}	30 day	90 day		Before	30 day	90 day
Control	Mean	2.3±1.5	3.0±2.1	4.3±1.6	1.5±0.7	55.2±11.7	56.3±9.0	61.8±7.9
	S.D.							
20	Mean	2.8±1.3	3.0±1.4	3.7±1.9	5.5±2.1	62.2±7.5	65.0±6.3	66.2±3.9
	S.D.							
100	Mean	2.0±2.1	1.8±1.0	3.8±1.9	6.5±2.1	66.8±10.5	59.0±11.9	63.3±4.1
	S.D.							
500	Mean	3.0±1.7	1.8±0.8	3.5±2.4	6.0±1.4	60.0±8.8	57.3±10.9	62.0±5.3
	S.D.							

a) : At the 5th day before starting b) : At the 30th day of withdrawal

表 28-5. Hematological findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	Before ^{a)}	Mono (%)		Recovery ^{b)}	Eosin (%)				
		30 day	90 day		Before	30 day	90 day		
Control	Mean S.D.	0.7±0.5	0.7±0.5	1.3±1.0	0.5±0.7	0.8±1.0	2.7±2.6	3.5±2.7	9.0±2.8
20	Mean S.D.	0.5±0.5	1.0±0.9	1.2±1.2	1.5±0.7	3.2±2.3	2.2±2.0	5.0±2.8	2.5±2.1
100	Mean S.D.	0.8±1.2	1.3±1.2	0.8±1.0	0.5±0.7	2.3±1.8	3.0±2.0	4.2±2.3	7.5±3.5
500	Mean S.D.	1.2±0.4	1.5±0.8	0.8±1.2	1.0±0.0	3.3±2.8	1.3±2.3	1.8±1.6	2.0±2.8

a) : At the 5th day before starting b) : At the 30th day of withdrawal

表 28-6. Hematological findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		Before ^{a)}			Baso (%)			Recovery ^{b)}			Ret (%)		
Control	Mean S.D.	0.0±0.0	0.0±0.0	0.2±0.4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.8±0.8	2.0±0.9	1.5±0.5	1.5±0.7	1.5±0.7
20	Mean S.D.	0.2±0.4	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	2.2±0.8	1.3±0.5	2.3±1.0	1.5±0.7	1.5±0.7
100	Mean S.D.	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.5±0.5	1.8±1.0	1.5±0.5	1.5±0.7	1.5±0.7
500	Mean S.D.	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.7±0.5	1.8±0.4	2.5±1.4	2.5±0.7	2.5±0.7

a) : At the 5th day before starting b) : At the 30th day of withdrawal

表 28-7. Hematological findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		PT (sec)		PTT (sec)	
		Before ^{a)}	30 day	90 day	Recovery
Control	Mean S.D.	8.4±0.5	9.0±1.8	8.8±1.2	8.0±0.6
20	Mean S.D.	8.4±0.5	10.5±3.7	8.8±2.5	10.1±2.8
100	Mean S.D.	8.7±0.7	10.7±4.0	9.9±2.5	10.0±2.5
500	Mean S.D.	8.0±0.4	8.4±0.6	8.9±0.4	8.3±0.1

a) : At the 5th day before starting b) : At the 30th day of withdrawal

表 28-8. Hematological findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		MCV (μ^3)		MCH ($\gamma\gamma$)	
		Before ^{a)}	30 day	90 day	Recovery
Control	Mean S.D.	65.0±4.0	69.7±1.7	66.7±2.4	68.4±2.6
20	Mean S.D.	69.4±3.3	69.8±2.6	67.0±2.7	67.4±0.8
100	Mean S.D.	67.6±3.9	71.4±2.7	67.7±1.8	69.9±2.6
500	Mean S.D.	67.4±2.6	68.7±4.0	67.2±2.3	69.2±0.8

a) : At the 5th day before starting b) : At the 30th day of withdrawal

* Significantly different from control : * (p<0.05)

表 28-9. Hematological findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		MCHC (%)	
		Before ^{a)}	30 day
Control	Mean S.D.	35.1±2.3	32.5±0.6
20	Mean S.D.	33.3±0.7	33.8±1.2
100	Mean S.D.	33.3±1.4	33.2±1.9
500	Mean S.D.	34.0±3.0	35.8±2.8

a) : At the 5th day before starting b) : At the 30th day of withdrawal

* Significantly different from control : * (p<0.05)

表 29. Parameter and abbreviation in myelogram

Parameter	Abbreviation
Myeloblast	MYBL
Promyelocyte	PMY
Metamyelocyte	MT
Neutrophilic myelocyte	MY-N
Neutrophil	N
Eosinophilic myelocyte	MY-E
Basophilic myelocyte	MY-B
Proerythroblast	PEB
Erythroblast	EB
Polychromatic erythroblast	POEB
Normoerythroblast	NEB
Lymphocyte	L
Monocyte	MO
Plasma cell	P
Reticulum cell	REC
Mast cell	MAC
Megakaryocyte	MK

表 30. Myelogram in male dogs treated orally with ONO-2235 for 90 days

Group	No. of animals	M						E			M/E Ratio	L	MO	P	REC	MAC	MK	
		MYBL	PMY	MT	MY-N	N	MY-E	MY-B	PEB	EB								POEB
Control	4	0.4	1.8	3.5	1.8	32.8	0.5	0.2	1.9	0.0	34.3	1.19	20.9	0.6	0.3	0.0	0.0	0.1
Mean		0.3	0.9	1.3	0.3	2.8	0.5	0.3	0.4	0.0	6.7	0.36	1.3	0.7	0.3	0.1	0.0	0.2
S.D.																		
20.000 (mg/kg)	4	0.3	1.3	5.5	5.0	21.2	1.3	0.3	1.4	0.3	34.0	1.02	26.9	1.8	0.1	0.0	0.0	0.2
Mean		0.6	1.1	3.8	5.5	7.6	1.1	0.3	0.5	0.4	6.8	0.31	5.4	2.0	0.2	0.1	0.0	0.2
S.D.																		
100.000 (mg/kg)	4	0.9	2.7	8.4	2.7	18.1	2.4	0.5	2.0	0.6	33.5	1.00	24.9	1.8	0.8	0.0	0.0	0.1
Mean		0.8	1.9	3.4	2.3	4.1	1.7	0.4	0.6	0.7	3.3	0.19	5.0	0.6	0.7	0.0	0.1	0.3
S.D.						*								*				
500.000 (mg/kg)	3	0.9	2.3	4.8	2.0	27.5	2.1	0.0	3.1	0.3	34.5	1.05	21.1	0.7	0.3	0.0	0.0	0.1
Mean		0.8	1.2	3.4	1.0	5.4	0.5	0.0	0.6	0.4	5.0	0.18	1.4	0.1	0.2	0.0	0.0	0.1
S.D.							*											

Significantly different from control : * (p<0.05) ** (p<0.01)

表 31. Myelogram in female dogs treated orally with ONO-2235 for 90 days

Group	No. of animals	M						E			POEB	M/E	M/E												
		MYBL	PMY	MT	MY-N	N	MY-E	MY-B	PEB	EB			NEB	Ratio	L	MO	P	REC	MAC	MK					
Control	4	0.5	1.4	7.8	0.5	32.7	2.4	0.0	1.4	0.3	30.0	1.49	20.4	1.2	0.8	0.0	0.0	0.1							
		0.3	1.6	4.3	0.3	4.9	1.1	0.1	0.6	0.1	6.7								0.37	2.1	0.8	0.5	0.0	0.0	0.2
20.000 (mg/kg)	4	0.6	1.0	4.5	1.1	30.8	2.0	0.0	2.8	0.4	32.6	1.13	22.7	0.5	0.4	0.0	0.0	0.2							
		0.8	0.4	1.6	0.3	2.1	1.8	0.1	1.5	0.2	2.0								0.15	3.0	0.4	0.3	0.1	0.0	0.2
100.000 (mg/kg)	4	0.9	1.4	6.6	1.8	30.7	1.6	0.1	2.8	0.6	33.1	1.19	18.5	1.0	0.4	0.0	0.0	0.1							
		0.5	0.8	1.7	1.7	2.0	0.9	0.2	1.3	0.2	3.5								0.11	5.3	0.3	0.3	0.0	0.0	0.2
500.000 (mg/kg)	4	0.5	1.7	7.1	1.7	27.3	2.0	0.0	2.5	0.3	32.4	1.20	22.1	1.1	0.7	0.0	0.0	0.1							
		0.4	1.1	5.1	1.3	1.7	1.9	0.0	0.8	0.3	7.1								0.42	2.8	1.0	0.6	0.0	0.0	0.1

Significantly different from control : * (p<0.05)

表 32. Myelogram in male dogs restored for 30 days after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	M								E			M/E						
		MYBL	PMY	MT	MY-N	N	MY-E	MY-B	PEB	EB	POEB	NEB	Ratio	L	MO	P	REC	MAC	MK
Control	2	0.3	1.2	2.6	2.6	25.6	0.3	0.2	2.9	0.3	34.8		0.86	28.4	0.4	0.1	0.0	0.0	0.5
		0.1	0.6	2.0	0.8	0.3	0.4	0.0	0.7	0.1	2.8		0.04	4.8	0.0	0.1	0.0	0.0	0.1
20.000 (mg/kg)	2	0.2	1.4	3.8	1.7	28.4	2.1	0.0	3.2	0.4	37.4		0.93	20.2	0.4	0.5	0.0	0.0	0.1
		0.3	1.1	2.8	1.8	0.0	3.0	0.0	2.3	0.3	6.5		0.31	5.9	0.3	0.4	0.0	0.0	0.1
						*													
100.000 (mg/kg)	2	1.2	0.9	3.3	2.4	30.1	2.3	0.0	2.8	0.0	37.3		1.01	18.2	0.5	0.5	0.0	0.0	0.4
		0.3	0.1	0.7	0.3	0.1	0.4	0.0	1.1	0.0	2.4		0.08	3.4	0.1	0.1	0.0	0.0	0.3
						*													
500.000 (mg/kg)	2	1.1	0.5	2.2	3.1	29.5	2.5	0.1	3.9	0.2	37.2		0.95	17.8	0.5	0.2	0.0	0.0	0.7
		0.1	0.1	0.8	1.0	3.3	1.0	0.1	0.7	0.3	2.0		0.13	1.4	0.1	0.3	0.0	0.0	0.1

Significantly different from control : * ($p < 0.05$)

表 33. Myelogram in female dogs restored for 30 days after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	M								E			M/E		L	MO	P	REC	MAC	MK
		MYBL	PMY	MT	MY-N	N	MY-E	MY-B	PEB	EB	POEB	NEB	Ratio							
Control	2	0.4	0.8	5.1	1.5	26.2	1.2	0.0	3.4	0.2	32.9	0.97	0.97	27.3	0.3	0.4	0.0	0.0	0.0	0.0
		0.3	0.3	0.4	0.7	2.3	1.4	0.0	0.0	0.3	5.2	0.06	0.06	7.8	0.4	0.0	0.0	0.0	0.0	0.0
20.000 (mg/kg)	2	0.1	0.4	3.0	5.7	36.2	0.9	0.0	2.0	0.5	26.5	1.60	1.60	24.0	0.6	0.1	0.0	0.0	0.0	0.0
		0.1	0.3	0.6	0.1	4.0	0.7	0.0	0.3	0.1	0.1	0.15	0.15	3.1	0.3	0.1	0.0	0.0	0.0	0.0
100.000 (mg/kg)	2	0.3	0.3	3.9	2.3	33.2	2.0	0.0	0.7	0.0	34.2	1.22	1.22	22.4	0.4	0.1	0.0	0.0	0.0	0.1
		0.1	0.4	0.4	0.7	11.0	0.6	0.0	0.1	0.0	3.1	0.39	0.39	6.8	0.6	0.1	0.0	0.0	0.0	0.1
500.000 (mg/kg)	2	0.2	0.2	7.4	1.5	29.8	2.2	0.2	1.8	0.3	32.5	1.20	1.20	23.3	0.1	0.2	0.0	0.0	0.0	0.1
		0.3	0.3	4.8	0.4	0.3	1.1	0.3	0.0	0.1	1.8	0.26	0.26	5.5	0.1	0.0	0.0	0.0	0.0	0.1

Significantly different from control : * ($p < 0.05$)

表 34-1. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		TP (g/dl)		A/G	
		Before ^{a)}	30 day	90 day	Recovery
Control	Mean S.D.	5.33±0.14	5.48±0.32	5.35±0.23	5.40±0.57
20	Mean S.D.	5.07±0.15	5.28±0.22	5.05±0.25	5.35±0.35
100	Mean S.D.	5.18±0.32	5.22±0.24	5.20±0.40	5.40±0.57
500	Mean S.D.	5.08±0.21	5.02±0.53	5.18±0.54	5.25±0.50

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05) ** (p<0.01)

表 34-2. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		T-Cho (mg/dl)		TG (mg/dl)	
		Before ^{a)}	30 day	90 day	Recovery
Control	Mean S.D.	164.5±25.2	142.0±28.0	131.5±18.2	130.5±24.7
20	Mean S.D.	162.0±38.6	147.5±40.6	127.0±31.5	92.5±31.8
100	Mean S.D.	146.0±16.9	127.5±25.3	120.7±27.5	118.5±26.2
500	Mean S.D.	130.2±24.3	133.7±14.8	133.2±24.3	119.5±31.8

a) : At the 5th day before starting b) : At the 30th day of withdrawal

表 34-3. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		PL (mg/dl)		GOT (mU/ml)	
		Before ^{a)}	30 day	90 day	Recovery
Control	Mean S.D.	323.2±39.8	277.7±53.1	273.8±32.2	286.0±58.0
20	Mean S.D.	311.7±63.6	288.2±72.3	277.7±55.2	217.0±53.7
100	Mean S.D.	286.3±29.0	254.8±41.6	266.3±51.7	260.5±37.5
500	Mean S.D.	321.3±138.5	263.5±48.4	287.0±52.5	257.0±49.5

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 34-4. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		GPT (mU/ml)			Recovery ^{b)}	Al-P (KA)			Recovery
		Before ^{a)}	30 day	90 day		Before	30 day	90 day	
Control	Mean S.D.	29.0±3.8	30.0± 6.3	38.8± 9.1	28.0± 7.1	9.2±1.6	7.3±1.2	6.3±1.3	3.8±0.1
20	Mean S.D.	28.7±5.5	27.0± 5.3	31.5± 5.9	34.0±14.1	8.6±0.9	6.9±0.8	5.4±0.9	3.8±1.1
100	Mean S.D.	30.5±4.8	34.8±11.7	36.5± 8.2	31.0± 8.5	8.2±1.9	6.2±1.2	4.9±1.3	4.0±0.4
500	Mean S.D.	31.7±7.5	105.3±71.9	245.0±156.4	28.5± 0.7	7.6±2.2	9.3±3.8	15.0±6.8	4.6±1.7

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 34-5. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	.	Glu (mg/dl)			Recovery ^{b)}	BUN (mg/dl)			
		Before ^{a)}	30 day	90 day		Before	30 day	90 day	
Control	Mean	104.8± 3.2	100.5±5.5	96.0±6.5	91.5±2.1	12.10±2.33	14.20±3.11	16.03±3.32	14.20± 0.42
	S.D.								
20	Mean	103.0± 6.5	100.5±8.8	97.5±5.2	96.0±0.0	12.12±2.55	14.10±3.68	14.73±3.91	26.50±17.54
	S.D.								
100	Mean	103.5±10.4	100.2±8.0	95.7±5.2	96.0±5.7	12.07±3.22	13.93±2.66	12.95±3.30	10.30± 1.98
	S.D.								
500	Mean	96.3±16.5	91.5±6.2	90.6±4.2	92.5±0.7	9.93±2.09	17.65±7.18	14.32±1.02	8.45± 0.49
	S.D.								

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 34-6. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	Cre (mg/dl)				UA (mg/dl)				
	Before ^{a)}	30 day	90 day	Recovery ^{b)}	Before	30 day	90 day	Recovery	
Control	Mean S.D.	0.70±0.13	0.77±0.08	0.83±0.19	0.70±0.14	0.30±0.00	0.35±0.05	0.47±0.14	0.30±0.14
20	Mean S.D.	0.75±0.05	0.77±0.12	0.78±0.07	0.90±0.00	0.33±0.08	0.23±0.08 [*]	0.33±0.05	0.35±0.07
100	Mean S.D.	0.80±0.41	0.73±0.08	0.73±0.08	0.70±0.14	0.32±0.04	0.28±0.07	0.48±0.10	0.40±0.00
500	Mean S.D.	0.77±0.14	0.62±0.13	0.58±0.08 [*]	0.70±0.00	0.28±0.04	0.37±0.16	0.52±0.13	0.30±0.00

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 34-7. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	T-Bil (mg/dl)		LDH (mU/ml)	
	Before ^{a)}	30 day	Before	30 day
Control	Mean S.D.	0.27±0.05 0.30±0.00	0.35±0.05 0.40±0.00	0.40±0.00 0.40±0.00
20	Mean S.D.	0.23±0.08 0.30±0.00	0.32±0.04 0.40±0.00	0.40±0.00 0.40±0.00
100	Mean S.D.	0.22±0.12 0.23±0.05	0.35±0.05 0.30±0.00	0.30±0.00 0.30±0.00
500	Mean S.D.	0.18±0.04 0.38±0.12	0.40±0.12 0.40±0.12	0.30±0.00 0.30±0.00

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05) ** (p<0.01)

表 34-8. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	Amy (C-U)	
	Before ^{a)}	30 day
Control	Mean S.D.	673.8±132.3 733.7±128.4
20	Mean S.D.	673.2±116.4 688.0±110.2
100	Mean S.D.	726.5±172.8 726.0±161.3
500	Mean S.D.	719.2±107.3 620.8±150.4

a) : At the 5th day before starting b) : At the 30th day of withdrawal

表 34-9. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	CPK (mU/ml)		Ca ²⁺ (mg/dl)	
	Before ^{a)}	30 day	Before	30 day
Control	Mean S.D.	50.8±11.1 46.2±10.5	9.38±0.35 10.27±0.27	10.07±0.24 10.20±0.42
20	Mean S.D.	62.2±14.5 57.2±16.2	9.12±0.16 10.17±0.34	9.83±0.22 10.45±0.07
100	Mean S.D.	58.2±12.1 49.3±11.7	9.13±0.83 10.12±0.23	9.72±0.12 10.10±0.14
500	Mean S.D.	52.0±23.5 53.7±29.2	9.02±0.65 9.63±0.77	9.68±0.74 10.05±0.21

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 34-10. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	P (mg/dl)		Recovery ^{b)}		Na ⁺ (mEq/l)		Recovery
	Before ^{a)}	30 day	90 day	Before	30 day	90 day	
Control	Mean S.D.	5.10±0.21	5.42±0.61	4.55±0.55	4.75±0.49	147.7±1.2	153.7±0.7
20	Mean S.D.	5.18±0.30	5.37±0.28	4.82±0.50	4.65±0.78	147.0±1.1	153.7±1.8
100	Mean S.D.	5.03±0.22	5.20±0.18	4.62±0.46	4.25±0.21	147.2±2.0	153.3±1.4
500	Mean S.D.	4.70±0.30	5.23±0.87	5.18±0.73	4.95±0.21	151.2±3.8	154.3±1.0

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 34-11. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	K ⁺ (mEq/l)		Recovery ^{b)}		Cl ⁻ (mEq/l)		Recovery
	Before ^{a)}	30 day	90 day	Before	30 day	90 day	
Control	Mean S.D.	5.13±0.30	5.16±0.24	4.85±0.39	4.99±0.21	110.50±2.59	112.50±2.12
20	Mean S.D.	4.99±0.17	4.84±0.16	4.66±0.15	4.77±0.09	109.50±2.17	113.00±0.00
100	Mean S.D.	5.11±0.16	4.99±0.26	4.83±0.17	4.98±0.26	108.83±2.04	112.50±2.12
500	Mean S.D.	4.84±0.17	4.66±0.31	4.66±0.24	5.20±0.02	112.83±1.84	114.00±1.41

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 34-12. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	Alb (%)		Recovery ^{b)}		α1-G (%)		Recovery
	Before ^{a)}	30 day	90 day	Before	30 day	90 day	
Control	Mean S.D.	52.28±2.65	55.68±3.16	56.95±2.40	54.50±0.42	8.30±0.91	6.95±1.63
20	Mean S.D.	54.17±3.42	57.28±2.86	55.80±3.83	56.25±5.30	7.88±1.70	5.45±1.34
100	Mean S.D.	54.15±2.11	55.18±3.68	52.80±4.26	50.80±3.68	7.45±1.30	7.70±1.84
500	Mean S.D.	53.98±3.81	53.67±3.28	49.56±5.52	49.90±3.82	7.53±1.55	7.70±0.71

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 34-13. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	α 2-G (%)				α 3-G (%)				
	Before ^{a)}	30 day	90 day	Recovery ^{b)}	Before	30 day	90 day	Recovery	
Control	Mean	5.08±0.67	4.58±0.30	4.85±0.21	4.70±0.42	11.15±0.99	10.23±0.55	8.72±0.92	8.95±0.07
	S.D.								
20	Mean	6.10±0.78	4.95±0.52	4.95±0.32	4.40±0.14	11.70±1.14	9.85±0.70	8.92±0.90	8.50±0.28
	S.D.	*							
100	Mean	5.80±0.43	5.00±0.65	4.95±0.59	4.70±0.42	11.35±1.30	9.63±1.06	9.43±1.30	10.20±0.42
	S.D.								
500	Mean	5.88±0.82	4.67±0.54	5.10±0.85	5.05±0.50	10.78±0.45	10.27±1.55	8.50±0.58	8.45±0.21
	S.D.								

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 34-14. Biochemical findings in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	β -G (%)				γ -G (%)				
	Before ^{a)}	30 day	90 day	Recovery ^{b)}	Before	30 day	90 day	Recovery	
Control	Mean S.D.	17.48±2.18	17.47±1.37	19.47±1.54	17.90±0.00	5.38±1.92	5.65±1.55	4.55±0.99	6.65±0.64
20	Mean S.D.	15.20±2.18	15.92±1.57	19.48±2.83	20.20±3.82	4.57±0.94	4.82±0.58	4.17±0.73	4.90±0.00
100	Mean S.D.	16.23±1.97	17.50±3.02	21.62±2.98	19.20±3.39	4.67±1.51	6.25±1.72	5.12±1.34	7.00±2.12
500	Mean S.D.	16.33±3.05	19.13±2.95	23.20±5.27	23.60±4.24	5.18±2.08	5.65±1.20	6.00±1.11	4.95±0.78

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 35-1. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		TP (g/dl)		Recovery ^{b)}	A/G	
		Before ^{a)}	30 day	90 day	Before	30 day
Control	Mean S.D.	5.35±0.33	5.53±0.18	5.32±0.17	5.50±0.28	5.50±0.28
20	Mean S.D.	5.13±0.23	5.30±0.35	5.18±0.12	5.50±0.14	5.50±0.14
100	Mean S.D.	5.30±0.32	5.37±0.23	5.25±0.33	5.10±0.14	5.10±0.14
500	Mean S.D.	5.40±0.20	5.43±0.20	5.45±0.47	5.45±0.50	5.45±0.50

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 35-2. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		T-Chol (mg/dl)		Recovery ^{b)}	TG (mg/dl)	
		Before ^{a)}	30 day	90 day	Before	30 day
Control	Mean S.D.	142.8±28.7	116.3±18.0	108.0±17.7	101.0±26.9	101.0±26.9
20	Mean S.D.	154.3±17.5	127.2±17.6	132.7±21.0	139.0±19.8	139.0±19.8
100	Mean S.D.	150.5±32.0	123.2±21.4	118.5±11.1	99.5±9.2	99.5±9.2
500	Mean S.D.	158.0±29.8	147.3±19.2	135.3±20.2	162.5±16.3	162.5±16.3

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 35-3. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)		PL (mg/dl)		Recovery ^{b)}	GOT (mU/ml)	
		Before ^{a)}	30 day	90 day	Before	30 day
Control	Mean S.D.	279.0±47.8	243.0±29.6	238.0±37.4	226.0±46.7	226.0±46.7
20	Mean S.D.	303.5±28.1	258.5±35.0	287.5±43.2	297.0±24.0	297.0±24.0
100	Mean S.D.	295.0±46.5	254.2±39.6	256.0±16.7	221.5±9.2	221.5±9.2
500	Mean S.D.	335.3±24.3	300.3±29.4	303.2±44.6	337.5±34.6	337.5±34.6

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 35-4. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	GPT (mU/ml)			AIP (KA)		
	Before ^{a)}	30 day	90 day	Before	30 day	90 day
Control	Mean S.D.	29.7±4.0	26.8±3.3	29.0±4.7	29.0±5.7	29.0±5.7
20	Mean S.D.	37.3±7.2	31.3±5.9	33.5±3.8	29.0±4.2	29.0±4.2
100	Mean S.D.	34.5±7.1	33.0±9.8	33.8±7.8	37.5±0.7	37.5±0.7
500	Mean S.D.	32.8±4.2	90.3±27.2 ^{**}	149.5±25.8 ^{**}	26.5±6.4	26.5±6.4

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05), ** (p<0.01)

表 35-5. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	Glu (mg/dl)			BUN (mg/dl)		
	Before ^{a)}	30 day	90 day	Before	30 day	90 day
Control	Mean S.D.	103.0±3.1	97.8±8.4	97.2±4.4	92.5±4.9	92.5±4.9
20	Mean S.D.	106.2±7.7	95.7±8.0	94.7±7.5	92.5±6.4	92.5±6.4
100	Mean S.D.	105.3±6.2	98.0±3.8	96.5±4.6	96.0±2.8	96.0±2.8
500	Mean S.D.	98.7±12.9	93.0±6.4	91.8±8.1	94.5±0.7	94.5±0.7

a) : At the 5th day before starting b) : At the 30th day of withdrawal

表 35-6. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	Cre (mg/dl)			UA (mg/dl)		
	Before ^{a)}	30 day	90 day	Before	30 day	90 day
Control	Mean S.D.	0.62±0.10	0.70±0.06	0.67±0.05	0.70±0.14	0.70±0.14
20	Mean S.D.	0.62±0.16	0.68±0.04	0.68±0.04	0.70±0.00	0.70±0.00
100	Mean S.D.	0.70±0.06	0.70±0.00	0.68±0.04	0.70±0.00	0.70±0.00
500	Mean S.D.	0.67±0.15	0.68±0.07	0.60±0.09	0.65±0.21	0.65±0.21

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 35-7. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	T-Bil (mg/dl)			LDH (mU/ml)					
	Before ^{a)}	30 day	90 day	Recovery ^{b)}	Before	30 day	90 day	Recovery	
Control	Mean S.D.	0.22±0.04	0.28±0.04	0.35±0.05	0.35±0.07	59.2±12.7	55.2±8.8	78.2±15.4	57.0±8.5
20	Mean S.D.	0.23±0.08	0.33±0.08	0.37±0.08	0.35±0.07	50.8±8.1	57.3±10.8	69.2±22.3	51.0±15.6
100	Mean S.D.	0.18±0.04	0.28±0.04	0.32±0.04	0.30±0.00	55.2±10.1	58.5±13.2	70.3±16.8	53.0±11.3
500	Mean S.D.	0.20±0.00	0.37±0.05 [*]	0.35±0.08	0.40±0.00	66.3±9.1	77.2±21.0	92.0±28.1	65.5±26.2

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 35-8. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	Amy (C-U)				
	Before ^{a)}	30 day	90 day	Recovery ^{b)}	
Control	Mean S.D.	627.8±162.3	713.7±115.6	733.5±124.6	759.5±20.5
20	Mean S.D.	681.5±184.3	707.7±165.4	752.8±204.1	766.5±285.0
100	Mean S.D.	709.8±130.7	715.2±129.7	725.7±208.2	766.5±251.0
500	Mean S.D.	672.0±45.3	609.7±54.9	675.3±54.3	610.5±70.0

a) : At the 5th day before starting b) : At the 30th day of withdrawal

表 35-9. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	CPK (mU/ml)					Ca ⁺⁺ (mg/dl)			
	Before ^{a)}	30 day	90 day	Recovery ^{b)}	Before	30 day	90 day	Recovery	
Control	Mean S.D.	103.2±62.5	52.8±19.8	54.2±12.4	40.5±4.9	8.98±0.83	10.33±0.20	9.77±0.33	10.10±0.00
20	Mean S.D.	68.5±21.0	52.5±19.7	44.7±9.5	36.0±4.2	8.95±0.46	10.23±0.42	9.88±0.40	10.55±0.07
100	Mean S.D.	79.5±24.0	51.7±7.3	46.2±10.0	42.5±10.6	8.92±0.59	10.22±0.55	9.52±0.45	10.00±0.71
500	Mean S.D.	77.0±12.5	51.2±20.4	40.2±11.2	46.5±26.2	9.07±0.44	9.87±0.34 [*]	9.45±0.29	10.15±0.35

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 35-10. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	P (mg/dl)			Na ⁺ (mEq/l)		
	Before ^{a)}	30 day	90 day	Before	30 day	90 day
Control	Mean S.D.	4.90±0.24	5.10±0.65	4.62±0.48	3.95±0.35	147.6±2.1
20	Mean S.D.	4.95±0.31	5.05±0.37	4.50±0.25	4.55±0.07	148.7±1.4
100	Mean S.D.	4.87±0.53	5.03±0.69	4.57±0.50	4.40±0.42	149.5±1.5
500	Mean S.D.	4.80±0.25	5.68±0.55	5.45±0.30	5.60±0.00	150.1±2.2

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * * (p<0.01)

表 35-11. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	K ⁺ (mEq/l)			Cl ⁻ (mEq/l)		
	Before ^{a)}	30 day	90 day	Before	30 day	90 day
Control	Mean S.D.	4.95±0.22	4.95±0.14	4.94±0.15	4.71±0.05	111.50±1.38
20	Mean S.D.	4.84±0.14	4.78±0.11	4.72±0.26	4.76±0.06	110.33±1.04
100	Mean S.D.	4.87±0.23	4.87±0.25	4.61±0.19	4.48±0.16	111.50±1.87
500	Mean S.D.	4.98±0.18	4.72±0.14	4.86±0.36	5.24±0.09	112.67±1.64

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05), * * (p<0.01)

表 35-12. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	Alb (%)			α1-G (%)		
	Before ^{a)}	30 day	90 day	Before	30 day	90 day
Control	Mean S.D.	53.05±4.91	54.15±2.47	52.97±2.62	49.25±2.90	8.20±1.31
20	Mean S.D.	57.35±1.89	51.18±4.68	53.87±2.72	51.40±1.84	7.62±0.80
100	Mean S.D.	56.35±2.52	54.70±3.72	51.30±4.63	53.15±0.78	6.88±1.41
500	Mean S.D.	54.30±3.62	52.67±1.84	48.32±3.87	47.80±3.25	7.72±1.94

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05)

表 35-13. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	Before ^{a)}	α 2-G (%)		Recovery ^{b)}	α 3-G (%)		Recovery
		30 day	90 day		30 day	90 day	
Control	Mean S.D.	5.83±0.81	4.90±0.30	4.62±0.41	5.75±0.92	10.50±2.50	8.48±1.13
20	Mean S.D.	5.88±0.85	5.20±0.42	3.98±0.45	5.25±0.35	10.17±1.21	8.33±1.20
100	Mean S.D.	5.60±0.91	5.27±0.54	4.55±0.41	5.45±0.07	10.70±0.87	8.20±0.85
500	Mean S.D.	5.25±0.42	5.60±0.50	6.60±0.59	5.25±1.06	11.33±0.33	8.43±1.49

a) : At the 5th day before starting b) : At the 30th day of withdrawal Significantly different from control : * (p<0.05), ** (p<0.01)

表 35-14. Biochemical findings in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	Before ^{a)}	β -G (%)		Recovery ^{b)}	γ -G (%)		Recovery
		30 day	90 day		30 day	90 day	
Control	Mean S.D.	16.57±2.30	19.25±2.43	19.55±3.40	23.25±5.45	5.57±1.51	6.60±1.13
20	Mean S.D.	14.20±2.07	17.60±3.13	17.12±2.45	19.25±0.78	4.47±1.13	5.65±0.35
100	Mean S.D.	14.98±2.01	17.47±2.66	19.97±5.09	18.70±1.84	5.20±0.84	8.15±1.34
500	Mean S.D.	15.97±1.97	19.48±3.16	22.92±3.34	21.90±4.81	5.13±0.75	6.80±0.99

a) : At the 5th day before starting b) : At the 30th day of withdrawal

表 36. BSP and PSP excretion tests in male dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	BSP (%)		PSP (%)	
	Before ^{a)}	Recovery ^{a)}	Before	Recovery
Control	Mean S.D.			
	1.6±0.6	1.7±0.7	70.3±5.6	99.4±18.3
20	Mean S.D.			
	1.0±0.2	1.4±0.8	64.6±6.6	83.4±16.2
100	Mean S.D.			
	1.6±0.3	1.4±0.2	69.7±16.0	85.1±18.5
500	Mean S.D.			
	1.1±0.4	7.1±5.2	51.4±12.5	111.4±21.7

a) : At the 5th day before starting b) : At the 30th day of withdrawal
Significantly different from control : * (p<0.05)

表 37. BSP and PSP excretion tests in female dogs treated orally with ONO-2235 for 90 days

Group (mg/kg)	BSP (%)		PSP (%)	
	Before ^{a)}	Recovery ^{a)}	Before	Recovery
Control	Mean S.D.			
	1.0±0.3	1.2±0.3	61.1±12.3	91.1±14.5
20	Mean S.D.			
	* 1.5±1.4	1.2±0.6	72.0±12.8	69.6±21.1
100	Mean S.D.			
	1.4±0.3	1.3±0.7	61.7±9.7	82.9±7.9
500	Mean S.D.			
	* 1.4±0.3	** 4.0±1.6	63.4±9.1	* 53.6±32.6

a) : At the 5th day before starting b) : At the 30th day of withdrawal
Significantly different from control : * (p<0.05), ** (p<0.01)

表 38-1. Absolute organ weights in male dogs treated orally with ONO-2235 for 90 days

Group	No. of animals	Brain (g)	Heart (g)	Lung (g)	Liver (g)	Kidney R(g)	Kidney L(g)	Spleen (g)	Pituitary (mg)	Thyroid (g)	Thymus (g)
Control	4	79.12	86.11	109.22	272.13	22.07	22.18	25.3	60.5	0.59	6.72
Mean		3.10	11.29	8.52	35.62	4.37	3.58	8.5	7.5	0.10	2.21
S.D.											
20.000 (mg/kg)	4	76.59	76.68	130.21	302.82	24.67	25.25	20.9	61.2	0.81	8.54
Mean		7.75	10.33	15.57	26.17	5.76	5.39	5.4	12.8	0.08	4.01
S.D.										*	
100.000 (mg/kg)	4	78.06	87.72	99.42	299.55	26.83	27.30	26.4	64.7	0.76	6.65
Mean		5.58	12.76	26.60	46.19	5.37	4.92	2.3	8.3	0.16	1.90
S.D.											
500.000 (mg/kg)	3	77.66	74.57	77.83	253.57	44.98	43.05	20.8	60.3	0.73	3.18
Mean		3.48	7.34	17.60	38.99	9.55	10.46	4.2	3.8	0.10	0.31
S.D.											

Significantly different from control : * (p<0.05)

表 38-2. Absolute organ weights in male dogs treated orally with ONO-2235 for 90 days

Group	No. of animals	Adrenal R(g)	Adrenal L(g)	Testis R(g)	Testis L(g)	Epididymis (g)	Prostate (g)	GLSubmand (g)
Control	4	0.57	0.52	8.15	8.53	3.66	4.62	10.05
Mean		0.09	0.08	0.66	1.31	0.38	1.42	1.76
S.D.								
20.000 (mg/kg)	4	0.52	0.54	6.68	6.46	3.18	4.32	13.24
Mean		0.08	0.10	3.03	2.61	0.50	3.15	2.43
S.D.								
100.000 (mg/kg)	4	0.55	0.53	7.42	7.10	3.20	5.32	11.19
Mean		0.08	0.10	1.72	1.81	0.63	2.12	1.40
S.D.								
500.000 (mg/kg)	3	0.61	0.57	5.21	5.10	2.47	1.48	9.31
Mean		0.09	0.01	0.58	0.27	0.28	0.79	3.29
S.D.				**	*	*	*	

Significantly different from control : * (p<0.05) ** (p<0.01)

表 39-1. Relative organ weights in male dogs treated orally with ONO-2235 for 90 days

Group	No. of animals	Brain (g)	Heart (g)	Lung (g)	Liver (g)	Kidney R(g)	Kidney L(g)	Spleen (g)	Pituitary (mg)	Thyroid (g)	Thymus (g)
Control											
Mean	4	8.21	8.91	11.29	28.30	2.27	2.30	2.6	6.3	0.06	0.69
S.D.		0.52	1.03	0.12	4.73	0.36	0.42	0.8	1.0	0.01	0.20
20.000 (mg/kg)											
Mean	4	7.82	7.81	13.36	31.08	2.49	2.55	2.1	6.3	0.08	0.84
S.D.		0.58	0.69	2.08	3.99	0.39	0.38	0.3	1.3	0.02	0.34
100.000 (mg/kg)											
Mean	4	8.06	8.96	10.09	30.82	2.74	2.80	2.7	6.6	0.08	0.70
S.D.		0.99	0.56	1.82	5.17	0.47	0.44	0.2	0.6	0.01	0.24
500.000 (mg/kg)											
Mean	3	10.13	9.59	10.33	32.52	5.93	5.68	2.6	7.8	0.09	0.41
S.D.		1.95	0.40	3.93	2.58	1.92	2.00	0.2	0.6	0.00	0.05

Significantly different from control : ** ($p < 0.01$)

表 39-2. Relative organ weights in male dogs treated orally with ONO-2235 for 90 days

Group	No. of animals	Adrenal R(g)	Adrenal L(g)	Testis R(g)	Testis L(g)	Epididymis (g)	Prostate (g)	GLSubmand (g)
Control								
Mean	4	0.06	0.05	0.84	0.88	0.38	0.48	1.04
S.D.		0.01	0.01	0.08	0.12	0.04	0.15	0.19
20.000 (mg/kg)								
Mean	4	0.05	0.05	0.68	0.66	0.32	0.43	1.35
S.D.		0.00	0.01	0.30	0.26	0.03	0.28	0.24
100.000 (mg/kg)								
Mean	4	0.06	0.05	0.76	0.72	0.33	0.54	1.14
S.D.		0.01	0.01	0.15	0.13	0.04	0.19	0.06
500.000 (mg/kg)								
Mean	3	0.08	0.07	0.68	0.66	0.32	0.19	1.17
S.D.		0.00	0.01	0.18	0.13	0.08	0.09	0.29

Significantly different from control : * ($p < 0.05$)

表 40-1. Absolute organ weights in female dogs treated orally with ONO-2235 for 90 days

Group	No. of animals	Brain (g)	Heart (g)	Lung (g)	Liver (g)	Kidney R(g)	Kidney L(g)	Spleen (g)	Pituitary (mg)	Thyroid (g)	Thymus (g)
Control	4	78.35	76.29	114.93	269.74	23.41	23.46	27.9	81.0	0.82	6.93
Mean		5.28	12.96	26.37	43.55	3.40	3.86	5.2	9.9	0.16	3.05
S.D.											
20.000 (mg/kg)	4	75.26	83.78	111.87	280.20	23.43	24.47	23.0	65.0	0.71	5.30
Mean		4.68	6.56	20.06	52.15	3.51	3.19	4.3	4.5	0.24	2.15
S.D.									*		
100.000 (mg/kg)	4	71.33	69.38	98.55	242.07	24.25	24.81	27.2	71.2	0.74	6.15
Mean		1.39	2.69	11.94	19.67	1.24	1.37	5.3	4.6	0.10	1.28
S.D.											
500.000 (mg/kg)	4	76.68	78.76	102.40	270.25	49.41	48.23	25.1	62.0	0.75	3.07
Mean		1.27	3.57	40.47	22.48	15.09	14.59	3.7	14.8	0.09	1.13
S.D.						*	*				

Significantly different from control : * ($p < 0.05$)

表 40-2. Absolute organ weights in female dogs treated orally with ONO-2235 for 90 days

Group	No. of animals	Adrenal R(g)	Adrenal L(g)	Ovary R(g)	Ovary L(g)	Uterus (g)	GLSubmand (g)
Control	4	0.69	0.62	0.35	0.45	2.12	11.83
Mean		0.25	0.21	0.12	0.13	0.72	2.23
S.D.							
20.000 (mg/kg)	4	0.68	0.69	0.42	0.46	2.24	11.24
Mean		0.03	0.09	0.15	0.14	0.80	1.79
S.D.							
100.000 (mg/kg)	4	0.56	0.58	0.54	0.54	5.48	10.45
Mean		0.12	0.09	0.29	0.32	8.49	1.69
S.D.							
500.000 (mg/kg)	4	0.68	0.59	0.48	0.49	2.31	12.13
Mean		0.16	0.09	0.16	0.14	2.32	3.45
S.D.							

表 41-1. Relative organ weights in female dogs treated orally with ONO-2235 for 90 days

Group	No. of animals	Brain (g)	Heart (g)	Lung (g)	Liver (g)	R(g)	Kidney L(g)	Spleen (g)	Pituitary (mg)	Thyroid (g)	Thymus (g)
Control	4	8.38	8.08	12.45	28.61	2.47	2.48	3.0	8.6	0.09	0.74
Mean		1.27	1.15	4.03	4.20	0.15	0.20	0.8	0.4	0.01	0.34
S.D.											
20.000 (mg/kg)	4	8.63	9.58	12.72	31.78	2.67	2.78	2.6	7.5	0.08	0.60
Mean		1.03	0.99	1.81	4.04	0.34	0.27	0.5	1.1	0.02	0.23
S.D.											
100.000 (mg/kg)	4	8.44	8.19	11.61	28.52	2.87	2.94	3.2	8.4	0.09	0.72
Mean		0.72	0.47	1.29	1.66	0.29	0.33	0.4	0.3	0.02	0.14
S.D.											
500.000 (mg/kg)	4	10.08	10.36	13.25	35.61	6.47	6.31	3.3	8.1	0.10	0.40
Mean		0.64	0.86	4.63	4.42	1.87	1.83	0.4	1.8	0.01	0.14
S.D.											

Significantly different from control : * ($p < 0.05$)

表 41-2. Relative organ weights in female dogs treated orally with ONO-2235 for 90 days

Group	No. of animals	Adrenal R(g)	Adrenal L(g)	Ovary R(g)	Ovary L(g)	Uterus (g)	GL.Submand (g)
Control	4	0.07	0.07	0.04	0.05	0.22	1.26
Mean		0.03	0.03	0.01	0.02	0.06	0.25
S.D.							
20.000 (mg/kg)	4	0.08	0.08	0.05	0.05	0.26	1.27
Mean		0.01	0.01	0.01	0.01	0.12	0.12
S.D.							
100.000 (mg/kg)	4	0.07	0.07	0.06	0.06	0.59	1.24
Mean		0.01	0.01	0.03	0.03	0.88	0.25
S.D.							
500.000 (mg/kg)	4	0.09	0.08	0.06	0.06	0.30	1.60
Mean		0.02	0.02	0.02	0.02	0.29	0.52
S.D.							

表 42-1. Absolute organ weights in male dogs restored for 30 days after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	Brain (g)	Heart (g)	Lung (g)	Liver (g)	Kidney R(g)	Kidney L(g)	Spleen (g)	Pituitary (mg)	Thyroid (g)	Thymus (g)
Control											
Mean	2	77.81	78.12	84.75	272.08	26.62	26.92	24.9	65.0	0.70	9.66
S.D.		2.60	0.85	23.53	11.91	3.59	2.53	6.7	9.9	0.03	0.55
20.000 (mg/kg)											
Mean	2	79.26	84.52	113.79	317.09	23.83	24.92	28.1	66.5	0.67	5.41
S.D.		2.20	1.36	37.96	77.84	1.27	1.73	1.4	2.1	0.13	0.14
100.000 (mg/kg)											
Mean	2	84.04	86.71	97.97	284.72	28.96	28.50	30.5	85.0	0.66	12.40
S.D.		11.58	9.82	4.53	22.09	3.27	3.63	4.4	12.7	0.08	6.08
500.000 (mg/kg)											
Mean	2	74.55	83.02	99.17	263.28	34.15	34.38	22.7	71.5	1.03	7.13
S.D.		0.77	9.16	12.48	48.99	1.71	0.52	4.5	2.1	0.29	2.00

表 42-2. Absolute organ weights in male dogs restored for 30 days after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	Adrenal R(g)	Adrenal L(g)	Testis R(g)	Testis L(g)	Epididymis (g)	Prostate (g)	GL Submand (g)
Control								
Mean	2	0.57	0.63	5.17	5.16	2.77	3.94	9.04
S.D.		0.06	0.00	1.97	1.80	0.06	1.49	0.57
20.000 (mg/kg)								
Mean	2	0.63	0.66	7.18	6.99	3.80	6.24	13.41
S.D.		0.00	0.07	0.40	0.48	0.20	0.58	1.11
100.000 (mg/kg)								
Mean	2	0.55	0.52	11.05	10.40	3.81	3.77	10.83
S.D.		0.07	0.05	0.54	1.03	0.13	1.52	0.58
500.000 (mg/kg)								
Mean	2	0.56	0.51	6.68	6.48	3.40	4.22	9.31
S.D.		0.08	0.02	0.18	0.27	0.35	1.56	0.57

表 43-1. Relative organ weights in male dogs restored for 30 days after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	Brain (g)	Heart (g)	Lung (g)	Liver (g)	Kidney R(g)	Kidney L(g)	Spleen (g)	Pituitary (mg)	Thyroid (g)	Thymus (g)
Control											
Mean	2	7.82	7.86	8.47	27.35	2.67	2.70	2.5	6.5	0.07	0.97
S.D.		0.13	0.48	1.94	0.16	0.23	0.12	0.5	0.7	0.00	0.10
20.000 (mg/kg)											
Mean	2	7.93	8.45	11.33	31.61	2.38	2.50	2.8	6.7	0.07	0.54
S.D.		0.44	0.10	3.48	6.89	0.19	0.24	0.1	0.4	0.02	0.00
100.000 (mg/kg)											
Mean	2	8.30	8.57	9.72	28.20	2.86	2.82	3.0	8.5	0.06	1.26
S.D.		0.45	0.25	0.37	0.18	0.08	0.12	0.7	2.0	-0.00	0.71
500.000 (mg/kg)											
Mean	2	8.03	8.87	10.59	27.96	3.67	3.70	2.4	7.7	0.11	0.75
S.D.		1.25	0.49	0.42	0.58	0.42	0.56	0.1	1.0	0.01	0.09

表 43-2. Relative organ weights in male dogs restored for 30 days after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	Adrenal R(g)	Adrenal L(g)	Testis R(g)	Testis L(g)	Epididymis (g)	Prostate (g)	GL-Submand (g)
Control								
Mean	2	0.06	0.06	0.52	0.51	0.28	0.40	0.91
S.D.		0.01	0.00	0.17	0.15	0.02	0.17	0.01
20.000 (mg/kg)								
Mean	2	0.06	0.07	0.72	0.70	0.38	0.63	1.34
S.D.		0.00	0.01	0.02	0.03	0.03	0.08	0.07
100.000 (mg/kg)								
Mean	2	0.05	0.05	1.10	1.03	0.38	0.37	1.08
S.D.		0.01	0.01	0.04	0.02	0.04	0.12	0.15
500.000 (mg/kg)								
Mean	2	0.06	0.05	0.72	0.70	0.36	0.44	1.01
S.D.		0.00	0.01	0.14	0.09	0.02	0.09	0.23

表 44-1. Absolute organ weights in female dogs restored for 30 days after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	Brain (g)	Heart (g)	Lung (g)	Liver (g)	Kidney R(g)	Kidney L(g)	Spleen (g)	Pituitary (mg)	Thyroid (g)	Thymus (g)
Control											
Mean	2	78.81	80.55	91.12	287.33	27.16	25.70	27.3	59.0	0.75	9.98
S.D.		4.12	3.15	4.91	3.06	1.01	0.92	0.6	4.2	0.23	2.86
20.000 (mg/kg)											
Mean	2	70.70	75.05	87.45	254.75	21.03	21.01	22.8	84.0	0.78	3.36
S.D.		1.77	6.38	33.05	22.63	1.01	1.69	2.4	36.8	0.10	0.85
100.000 (mg/kg)											
Mean	2	70.13	78.21	110.43	245.50	23.15	24.05	25.3	78.0	0.66	2.50
S.D.		3.62	3.42	4.38	51.10	2.62	2.61	2.0	5.7	0.18	0.69
500.000 (mg/kg)											
Mean	2	72.61	78.42	76.01	276.43	26.30	27.59	22.3	68.5	0.57	11.58
S.D.		10.07	4.29	3.19	11.03	0.42	2.80	8.2	7.8	0.16	5.67

表 44-2. Absolute organ weights in female dogs restored for 30 days after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	Adrenal R(g)	Adrenal L(g)	Ovary R(g)	Ovary L(g)	Uterus (g)	GL.Submand (g)
Control							
Mean	2	0.53	0.55	0.44	0.48	1.45	11.40
S.D.		0.07	0.01	0.03	0.06	0.03	1.51
20.000 (mg/kg)							
Mean	2	0.44	0.42	1.24	0.98	15.30	9.86
S.D.		0.03	0.03	0.28	0.34	1.86	0.08
100.000 (mg/kg)							
Mean	2	0.54	0.55	0.43	0.48	1.76	10.42
S.D.		0.08	0.06	0.05	0.16	0.18	0.17
500.000 (mg/kg)							
Mean	2	0.58	0.57	0.80	1.18	6.75	12.19
S.D.		0.05	0.01	0.74	1.17	6.50	2.84

表 45-1. Relative organ weights in female dogs restored for 30 days after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	Brain (g)	Heart (g)	Lung (g)	Liver (g)	Kidney R(g)	Kidney L(g)	Spleen (g)	Pituitary (mg)	Thyroid (g)	Thymus (g)
Control											
Mean	2	8.31	8.53	9.61	30.38	2.87	2.72	2.9	6.2	0.08	1.04
S.D.		0.31	1.09	0.34	3.04	0.15	0.34	0.3	0.1	0.03	0.21
20.000 (mg/kg)											
Mean	2	7.76	8.21	9.42	27.86	2.30	2.30	2.5	9.0	0.08	0.37
S.D.		0.59	0.13	2.66	0.32	0.12	0.05	0.0	3.1	0.00	0.13
100.000 (mg/kg)											
Mean	2	8.08	9.01	12.69	28.40	2.67	2.77	2.9	9.0	0.08	0.28
S.D.		0.81	0.83	0.12	7.26	0.43	0.43	0.1	1.1	0.02	0.07
500.000 (mg/kg)											
Mean	2	8.47	9.17	8.92	32.35	3.08	3.22	2.6	8.0	0.07	1.34
S.D.		0.69	0.03	0.89	0.58	0.13	0.14	0.8	0.4	0.02	0.59

表 45-2. Relative organ weights in female dogs restored for 30 days after the cessation of oral administration of ONO-2235 for 90 days

Group	No. of animals	Adrenal R(g)	Adrenal L(g)	Ovary R(g)	Ovary L(g)	Uterus (g)	GL Submand (g)
Control							
Mean	2	0.06	0.06	0.05	0.05	0.15	1.21
S.D.		0.01	0.00	0.01	0.01	0.01	0.27
20.000 (mg/kg)							
Mean	2	0.05	0.05	0.14	0.10	1.67	1.08
S.D.		0.00	0.00	0.04	0.03	0.03	0.10
100.000 (mg/kg)						*	
Mean	2	0.06	0.06	0.05	0.06	0.20	1.20
S.D.		0.01	0.01	0.01	0.02	0.03	0.08
500.000 (mg/kg)							
Mean	2	0.07	0.07	0.09	0.13	0.77	1.44
S.D.		0.00	0.01	0.08	0.13	0.72	0.41

Significantly different from control : * ($p < 0.05$)

表 46-1. Histopathological findings in male dogs treated orally with ONO-2235 for 90 days

Histopathological findings	Control						20 (mg/kg)						100 (mg/kg)						500 (mg/kg)						
	Termination			Rec ^{a,b}	Termination			Rec	Termination			Rec	Termination			Rec	Termination			Rec					
	1	3	5		6	2	4		7	8	11		12	9	10		13	14	16		18	15	17	19	21*
Kidney (Left and right)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	±	±	-	-
Degeneration and necrosis of proximal tubular cells	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	±	±	-	-
Dilation of lumina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	±	±	-	-
Casts in lumina	-	±	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	±	±	-	-
Loss of brush border (PAS-stain)	NS	NS	NS	NS	-	-	NS	NS	NS	NS	-	-	-	-	-	-	-	-	-	+	+	±	±	-	-
Vacuolar degeneration of proximal tubular cells (pars recta)	-	±	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Small nodule with foamy cells	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	± ^{b)}	± ^{b)}	-	-	-	-	-	-
Cell infiltrations	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Congestion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Heart (Left and right ventricle, and septum)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lung (Left and right lobes)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Focal pneumonia with perivascularitis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Granuloma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Appearances of <i>Filaroides hirthi</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trachea	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cell infiltrations	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Larynx	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Esophagus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tongue	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stomach	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fundus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pylorus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Small intestine (Duodenum, Jejunum and Ileum)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dilation of crypt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Large intestine (Colon, Cecum and Rectum)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* : Dead case a) : Rec; Recovery b) : In right side NS : No staining Grading : - No remarkable change. ± Very slightly. + Slightly

表 46-2. Histopathological findings in male dogs treated orally with ONO-2235 for 90 days

Histopathological findings	Control						20 (mg/kg)						100 (mg/kg)						500 (mg/kg)					
	Termination 1 3 5 6	Rec ^{a)} 2 4	Termination 7 8 11 12	Rec 9 10	Termination 13 14 16 18	Rec 15 17	Termination 19 21 22 23	Rec 20 24	Termination 21 22 23	Rec 20 24	Termination 21 22 23	Rec 20 24	Termination 21 22 23	Rec 20 24	Termination 21 22 23	Rec 20 24	Termination 21 22 23	Rec 20 24	Termination 21 22 23	Rec 20 24	Termination 21 22 23	Rec 20 24	Termination 21 22 23	Rec 20 24
Liver (External left and right, internal right lobes)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brown pigment deposition in Kupffer cells	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Centrilobular congestion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clearing of hepatocytes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cell infiltrations in Glisson's sheath	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pancreas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Submandibular gland	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thoracic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abdominal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pituitary	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Decreases of colloid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Desquamation of follicular epithelium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cell infiltrations	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Parathyroid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adrenal (Left and right)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Congestion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urinary bladder	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemorrhages in epithelium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Testis (Left side)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Disorder of spermatogenesis, focally	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Appearances of polynuclea gaint cells	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Epididymis (Left side)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
No appearances of sperm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Atrophy of glandular epithelium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prostate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hypoplasia of gland	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* : Dead case a) : Rec; Recovery c) : In external right lobe d) : And proliferation of interstitium ND : Not detectable
 Grading : - No remarkable change, ± Very slightly, + Slightly

表 46-3. Histopathological findings in male dogs treated orally with ONO-2235 for 90 days

Histopathological findings	Control						20 (mg/kg)						100 (mg/kg)						500 (mg/kg)					
	Termination 1	3	5	6	Rec ^{a)} 2	4	Termination 7	8	11	12	Rec 9	10	Termination 13	14	16	18	Rec 15	17	Termination 19	21 ^{b)}	22	23	Rec 20	24
Hemorrhages in lymph follicle	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Hemosiderosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	±	-	-	-	-
Spleen	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Atrophy of white pulps	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Congestion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thymus	-	-	-	-	-	-	+	-	-	±	-	-	-	-	-	-	-	-	±	±	±	-	-	-
Submandibular lymph node	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemosiderosis	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Decreases of lymphocytes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mesenteric lymph node	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Decreases of lymphocytes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Capsulitis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Congestion	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tonsilla	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Focal necrosis of squamous epithelium	-	± ⁿ⁾	-	±	-	±	±	-	-	-	±	±	-	-	-	-	-	-	± ⁿ⁾	-	-	-	±	-
Hemorrhages	-	-	-	-	-	-	-	-	-	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-
Hemosiderosis	-	±	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Calcification	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Congestion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bone marrow	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* : Dead case a) : Rec; Recovery e) : In perivascular connective tissue f) : In submucosal region
Grading : - No remarkable change, ± Very slightly, + Slightly, ± Moderately, ± Strongly

表 46-4. Histopathological findings in male dogs treated orally with ONO-2235 for 90 days

Histopathological findings	Control						20 (mg/kg)						100 (mg/kg)						500 (mg/kg)							
	Termination			Rec ^a	Termination			Rec	Termination			Rec	Termination			Rec	Termination			Rec	Termination			Rec		
	1	3	5		6	2	4		7	8	11		12	9	10		13	14	16		18	15	17		19	21*
Cerebrum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cerebellum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Medulla oblongata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Spinal cord	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sciatic nerve	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Eye (Optic nerve, Retina and Iris)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cell infiltrations in conjunctiva	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dermatitis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Granuloma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±
Mammary gland	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Atrophy of gland	ND	ND	ND	ND	ND	ND	+	+	ND	ND	ND	ND	ND	ND	ND	ND	+	+	ND	+	+	+	+	+	ND	+
Proliferation of gland	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* : Dead case a) : Rec; Recovery ND : Not detectable

表 47-1. Histopathological findings in female dogs treated orally with ONO-2235 for 90 days

Histopathological findings	Control			20 (mg/kg)			100 (mg/kg)			500 (mg/kg)		
	Termination	Rec ^{a)}		Termination	Rec		Termination	Rec		Termination	Rec	
Kidney (Left and right)	501 504 505 506	502 503		507 508 509 511	510 512		514 516 517 518	513 515		519 521 522 524	520 523	
Degeneration and necrosis of proximal tubular cells	-	-	-	-	-	-	-	-	-	±	±	-
Dilation of lumina	-	-	-	+	-	-	-	-	-	±	±	-
Casts in lumina	-	-	-	-	-	-	-	-	-	-	-	-
Loss of brush border (PAS stain)	NS NS NS NS	-	-	NS NS NS NS	-	-	-	-	-	+	+	-
Vacuolar degeneration of proximal tubular cells (pars recta)	-	-	-	± ±	±	+	-	+	-	-	-	-
Small nodule with foamy cells	-	-	-	-	-	-	-	-	-	-	-	-
Cell infiltrations	-	-	-	-	-	-	-	-	-	+	-	-
Congestion	-	-	-	-	-	-	-	-	-	-	-	-
Heart (Left and right ventricle, and septum)	-	-	-	-	-	-	-	-	-	-	-	-
Lung (Left and right lobes)	-	-	-	-	-	-	-	-	-	-	-	-
Focal pneumonia with perivascularitis	-	-	-	-	-	-	-	-	-	-	-	-
Granuloma	-	-	-	-	-	-	-	-	-	-	± ^{a)}	-
Appearances of <i>Filaroides hirthi</i>	-	-	-	-	-	-	-	-	-	-	-	-
Trachea	-	-	-	-	-	-	-	+	-	-	-	-
Larynx	-	±	-	-	-	-	-	-	-	-	-	-
Esophagus	-	-	-	-	-	-	-	-	-	-	-	-
Tongue	-	-	-	-	-	-	-	-	-	-	-	-
Stomach	-	-	-	-	-	-	-	-	-	-	-	-
Fundus	-	-	-	-	-	-	-	-	-	-	-	-
Pylorus	-	-	-	-	-	-	-	-	-	-	-	-
Small intestine (Duodenum, Jejunum and Ileum)	-	-	-	-	-	-	-	-	-	-	-	-
Dilation of crypt	-	-	-	-	-	-	-	-	-	-	-	-
Large intestine (Colon, Cecum and Rectum)	-	-	-	-	-	-	-	-	-	-	-	-

a) : Rec; Recovery b) : In medulla of right kidney c) : In pelvis d) : In right lobe NS : No staining
Grading : - No remarkable change, ± Very slightly, + Slightly

表 47-2. Histopathological findings in female dogs treated orally with ONO-2235 for 90 days

Histopathological findings	Control				20 (mg/kg)				100 (mg/kg)				500 (mg/kg)			
	Termination 501 504 505 506	Rec ^{a)} 502 503	Termination 507 508 509 511	Rec 510 512	Termination 514 516 517 518	Rec 513 515	Termination 519 521 522 524	Rec 520 523	Termination 514 516 517 518	Rec 513 515	Termination 519 521 522 524	Rec 520 523	Termination 519 521 522 524	Rec 520 523	Termination 519 521 522 524	Rec 520 523
Liver (External left and right, internal right lobes)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Brown pigment deposition in Kupffer cells	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Centrilobular congestion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clearing of hepatocytes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cell infiltrations in Glisson's sheath	-	± ^{a)}	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pancreas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Submandibular gland	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thoracic	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abdominal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pituitary	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cyst formation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Decreases of colloid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thyroid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Desquamation of follicular epithelium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cell infiltrations	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Parathyroid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adrenal (Left and right)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Congestion	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urinary bladder	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hemorrhages in epithelium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ovary (Left side)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Follicle only	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Appearances of corpus luteum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lutein cyst	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Uterus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Vagina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

a) : Rec; Recovery e) : In external and internal right lobes f) : And hypertrophy of Kupffer cells g) : In external left and right lobes h) : In external left lobe
 ND : Not detectable Grading : - No remarkable change, ± Very slightly, + Slightly

表 47-3. Histopathological findings in female dogs treated orally with ONO-2235 for 90 days

Histopathological findings	Control			20 (mg/kg)			100 (mg/kg)			500 (mg/kg)		
	Termination 501 504 505 506	Rec ^{a)} 502 503 506		Termination 507 508 509 511	Rec 510 512		Termination 514 516 517 518	Rec 513 515		Termination 519 521 522 524	Rec 520 523	
Hemorrhages in lymph follicle	-	-		-	-		-	-		-	-	
Spleen	-	-		-	-		-	-		-	-	
Hemosiderosis	-	-		-	-		-	-		-	-	
Atrophy of white pulps	-	-		-	-		-	-		-	-	
Thymus	-	-		-	-		-	-		-	-	
Submandibular lymph node	-	-		-	-		-	-		-	-	
Hemosiderosis	-	-		-	-		-	-		-	-	
Decreases of lymphocytes	-	-		-	-		-	-		-	-	
Mesenteric lymph node	-	-		-	-		-	-		-	-	
Decreases of lymphocytes	-	-		-	-		-	-		-	-	
Capsulitis	-	-		-	-		-	-		-	-	
Congestion	-	-		-	-		-	-		-	-	
Tonsilla	-	-		-	-		-	-		-	-	
Focal necrosis of squamous epithelium	-	-		-	-		-	-		-	-	
Hemorrhages	-	-		-	-		-	-		-	-	
Hemosiderosis	-	-		-	-		-	-		-	-	
Calcification	-	-		-	-		-	-		-	-	
Congestion	-	-		-	-		-	-		-	-	
Bone marrow	-	-		-	-		-	-		-	-	

a) : Rec, Recovery i) : In submucosal region Grading : - No remarkable change, ± Very slightly, + Slightly, # Moderately, ## Strongly

表 47-4. Histopathological findings in female dogs treated orally with ONO-2235 for 90 days

Histopathological findings	Control			20 (mg/kg)			100 (mg/kg)			500 (mg/kg)		
	Termination 501 504 505 506	Rec ^{a)} 502 503		Termination 507 508 509 511	Rec 510 512		Termination 514 516 517 518	Rec 513 515		Termination 519 521 522 524	Rec 520 523	
Cerebrum	-	-		-	-		-	-		-	-	
Cortex	-	-		-	-		-	-		-	-	
Thalamus	-	-		-	-		-	-		-	-	
Hypothalamus	-	-		-	-		-	-		-	-	
Cerebellum	-	-		-	-		-	-		-	-	
Medulla oblongata	-	-		-	-		-	-		-	-	
Spinal cord	-	-		-	-		-	-		-	-	
Sciatic nerve	-	-		-	-		-	-		-	-	
Eye (Optic nerve, Retina and Iris)	-	-		-	-		-	-		-	-	
Cell infiltrations in conjunctiva	-	-		-	-		-	-		-	-	
Dermatitis	-	-		-	-		-	-		-	-	
Skin	-	-		-	-		-	-		-	-	
Granuloma	-	-		-	-		-	-		-	-	
Mammary gland	-	-		-	-		-	-		-	-	
Atrophy of gland	-	-		-	-		-	-		-	-	
Proliferation of gland	-	-		-	-		-	-		-	-	

a) : Rec; Recovery ND : Not detectable

下でも認められ²⁾、可逆的な機能的適応によるものと考えられる³⁻⁵⁾ことから、病的変化を示す特異的所見ではなく、ONO-2235 投与による機能的適応を反映した結果であろうと考えられた。

心電図検査で100mg/kg 以上の投与群で心拍動数の減少傾向がみられ、心機能に対する影響が考慮されたが、著明な異常所見は得られなかった。

その他、剖検で500mg/kg 投与群の雄1例に胃粘膜の白斑、対照群の雌1例に胃噴門部に出血点が認められたが、病理組織学的に明確にし得なかった。しかしその発現頻度は低く、検体投与との因果関係はないものと考えられる。

以上の如く、ONO-2235 の20, 100, 500mg/kg をビーグル犬に90日間経口投与した結果、100mg/kg 投与群で体重の減少、心拍動数の減少を認めた

が、形態学的に変化はなく、500mg/kg 投与群ではさらに肝臓および腎臓に形態的变化が認められたことより、確実中毒量は500mg/kg、無影響量は20 mg/kg と推察される。

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Effects of a fructose-rich diet and the aldose reductase inhibitor, ONO-2235, on the development of diabetic neuropathy in streptozotocin-treated rats

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Summary. Streptozotocin-diabetic rats were maintained on a 72% fructose diet for 4 weeks and some were treated with an aldose reductase inhibitor (either alrestatin: $0.9 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ or ONO-2235: $50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). Fructose feeding significantly influenced the development of impaired motor nerve conduction velocity in the diabetic rats and this effect was positively correlated with sorbitol accumulation in the sciatic nerve of diabetic rats maintained on a fructose-rich diet. Treatment with ONO-2235, a new aldose reductase inhibitor, prevented both slowing of motor nerve conduction velocity and elevation of nerve sorbitol concentration. On the other

hand, erythrocyte sorbitol levels were significantly correlated to those of the sciatic nerve ($r=0.86$, $p<0.001$) and the retina ($r=0.91$, $p<0.001$) in these animals. Thus, our findings suggest that increased polyol pathway activity may be related to the pathogenesis of diabetic neuropathy and erythrocyte sorbitol concentrations may prove a useful indicator for the presence of diabetic complications.

Key words. Fructose-rich diet, aldose reductase inhibitor, ONO-2235, diabetic neuropathy, polyol pathway, sorbitol, sciatic nerve, retina, erythrocyte.

Recent studies in animals [1-7], and to some extent in man [8-12], suggest that alterations of polyol pathway activity may play an important role in the development of diabetic neuropathy. However, as yet, there is no definitive evidence. As both genetically selected [13] and streptozotocin-diabetic rats [2, 14] maintained on a fructose-rich diet have been shown to develop diabetic angiopathy, it would seem that the fructose model in diabetic rats may provide new support for the role of the polyol pathway in the genesis of diabetic complications [15]. Moreover, sorbitol and fructose accumulation in the tissues of streptozotocin-diabetic rats maintained on a 72% fructose diet for 4 weeks, was 30% greater than in those maintained on a 72% glucose diet for the same period [16]. Thus, it was expected that fructose feeding to diabetic rats would result in the development of diabetic neuropathy, as does galactose feeding [1, 15, 17].

The aim of the present study was to elucidate the possible role of the polyol pathway in the aetiology of diabetic neuropathy and the beneficial effect of ONO-2235 on the prevention of peripheral neuropathy in diabetes mellitus.

Materials and methods

Animals and experimental protocols

Diabetes was induced in male Wistar rats (weight 200-250 g) by a low dose of streptozotocin (40 mg/kg body weight). A single injection at a level not directly toxic to peripheral nerve was made into the tail vein

of rats fasted overnight. The drug was dissolved in saline (0.15 mol/l) immediately before injection.

After 2 weeks of streptozotocin administration, the diabetic rats (blood glucose $>20 \text{ mmol/l}$) were selected at random and divided into five groups. A control group (group D) had free access to laboratory chow and water without treatment for 4 weeks. The remaining four groups of rats were maintained on a 72% fructose-diet for 4 weeks as reported previously [3, 14]. One of these groups received no further treatment (group DF), whilst the others were treated with aldose reductase inhibitor [alrestatin (group DFA) or ONO-2235 (group DFO)] or insulin (group DFI). Aldose reductase inhibitor was added to the fructose diet. Alrestatin (a gift from Nihon-Kayaku, Tokyo, Japan) was fed as 0.7% of diet (approximately $0.9 \text{ g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) and (E)-3-carboxymethyl-5-[(E)-2-methyl-3-phenylpropenylidene] rhodanine (ONO-2235, a gift from Ono Pharmaceuticals, Osaka, Japan) was fed as 0.04% of the diet (approximately $50 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). Lente insulin was injected subcutaneously daily for 4 weeks at doses varying from 2 to 6 U according to the levels of glucose and ketone revealed in the urine by the reagent strips of Multistix (Miles-Sankyo, Tokyo, Japan). One group of normal rats was maintained on a 72% fructose diet for 4 weeks (group CF), whilst a control group (group C) had free access to laboratory chow and water only.

Measurement of motor nerve conduction velocity and analysis

Motor nerve conduction velocity (MNCV) was measured in the most rapidly conducting fibres of the rat tail nerve supplying the segmental muscle according to the method of Miyoshi and Goto [17]. The rats were kept on a heated pad in a room maintained at 25°C to preserve a constant rectal temperature of 37°C . After intraperitoneal injection of sodium pentobarbital ($30-40 \text{ mg/kg}$ body weight), MNCV was determined using a Neuropak NEM-3102 instrument (Nihon-Koden, Osaka, Japan) at 0, 2 and 4 weeks following the initiation of treatment.

Table 1. Body weight and blood glucose concentrations in rats maintained on specified treatment for 4 weeks

Animal group	Group code	Diet	Body weight (g)	Blood glucose (mmol/l)
Normal control rats	C	Laboratory chow (<i>n</i> = 10)	363 ± 8	5.78 ± 0.16
	CF	Fructose (<i>n</i> = 12)	360 ± 5	6.11 ± 1.02
Diabetic control rats	D	Laboratory chow (<i>n</i> = 7)	229 ± 18 ^{a,b}	10.00 ± 1.06 ^{a,b}
	DF	Fructose (<i>n</i> = 8)	219 ± 9 ^{a,b}	11.22 ± 0.56 ^{a,b}
Treated diabetic rats	DFA	Fructose (<i>n</i> = 6)	245 ± 32 ^{a,b}	9.56 ± 0.83 ^{a,b}
	DFO	Fructose (<i>n</i> = 7)	233 ± 12 ^{a,b}	10.56 ± 0.56 ^{a,b}
	DFI	Fructose (<i>n</i> = 7)	280 ± 7 ^{a,b,c,d,e}	10.50 ± 0.78 ^{a,b}

Results are expressed as mean ± SEM. ^a *p* < 0.001 versus group C; ^b *p* < 0.001 versus group CF; ^c *p* < 0.05 versus group D; ^d *p* < 0.001 versus group DF; ^e *p* < 0.01 versus group DFO rats

Table 2. Effect of a fructose-rich diet on motor nerve conduction velocity in streptozotocin-diabetic rats

Animal group	Group code	Motor nerve conduction velocity (m/s) at:		
		0 weeks	2 weeks	4 weeks
Normal rats				
	Untreated (<i>n</i> = 10)	29.2 ± 0.5	32.2 ± 1.5	35.4 ± 1.4
Fructose-fed (<i>n</i> = 12)	CF	29.4 ± 0.7	31.8 ± 1.4	36.0 ± 0.8
Diabetic rats				
	Untreated (<i>n</i> = 7)	27.9 ± 0.8	28.5 ± 0.7	32.2 ± 0.7 ^{a,c}
Fructose-fed (<i>n</i> = 8)	DF	27.3 ± 0.6 ^{a,f}	29.5 ± 0.7	28.2 ± 0.2 ^{a,d,e}

Results are expressed as mean ± SEM. ^{a,b,c} *p* < 0.001, 0.01, 0.05 versus group C; ^{d,e,f} *p* < 0.001, 0.01, 0.05 versus group CF respectively; ^e *p* < 0.001 versus group D rats

At the end of this experimental period, rats were fasted overnight before study. After induction of anaesthesia (sodium pentobarbital 30–40 mg/kg body weight), blood was drawn from the vena cava inferior, and collected in ice-cold tubes for determination of glucose and sorbitol. The sciatic nerves and retina were removed immediately. The sciatic nerves were removed from the level of the sciatic notch and extending approximately 3 cm distally. After removal of fat and connective tissues, the nerve was weighed, immediately frozen in liquid nitrogen and stored at –80°C until analysis for glucose and sugar alcohol contents.

To determine glucose, sorbitol and fructose concentrations, the nerves were homogenized in ice-cold 10% (wt/vol) HClO₄ (0.5 ml) in a glass-in-glass hand homogenizer (Kinoshita-Rika, Nagoya, Japan) and centrifuged at 1400 g for 10 min at 4°C. The supernatants were neutralized with 2N KOH. After centrifugation, glucose [18], sorbitol [19] and fructose [20] were measured enzymatically.

Sorbitol levels in the retina were measured as described above after homogenization in ice-cold 10% (wt/vol) HClO₄ (1.0 ml) and centrifugation at 1400 g for 10 min at 4°C. Erythrocyte sorbitol content was measured according to the technique of Malone et al. [21]. Blood glucose was determined using a glucose test kit (New Glucostat, Worthington Biochemicals, Freehold, New Jersey, USA).

Statistical methods

All results are presented as mean ± SEM. The significance of differences, were calculated by the Student's *t*-test.

Results

The changes in body weight for all groups of rats are shown in Table 1. Body weight and blood glucose levels in normal rats were similar in the fructose-fed group (group CF) and the laboratory chow-fed group (group C). The streptozotocin-diabetic rats lost weight significantly, but treatment with aldose reductase inhibitors (groups DFA, DFO) had no effects on weight loss or severity of hyperglycaemia. Differences in blood glucose levels between the insulin-treated diabetic group (group DFI) and the other diabetic groups were of little significance since the insulin dose was varied according to the diabetic condition of individual rats. However, group DFI rats did not lose weight significantly compared with the other groups of diabetic rats.

Effect of a fructose-rich diet and aldose reductase inhibitors on MNCV

The fructose feeding for 4 weeks significantly influenced the development of impaired MNCV in diabetic rats compared with untreated diabetic rats (28.2 ± 0.2 versus 32.2 ± 0.7 m/s, *p* < 0.001; Table 2). Sorbitol and fructose concentrations in the sciatic nerves were more markedly elevated in group DF than in group D rats (Table 3), suggesting that the MNCV defect may be related to nerve sorbitol and fructose contents. However, in normal rats, neither MNCV nor the concentrations of nerve glucose, sorbitol or fructose were affected by fructose feeding.

Table 4 shows the effects of aldose reductase inhibitors and insulin on the development of impaired MNCV in diabetic rats maintained on a fructose-rich diet. Animals in groups DFA, DFO, DFI showed a marked improvement in MNCV after 4 weeks, compared with those in group DF, but there were no differences in improvement of MNCV between the three treated diabetic groups. Sorbitol and fructose contents in the sciatic nerve were reduced by >40% in groups DFA, DFO, DFI compared with group DF rats (Table 5). However, since this reduction was only ap-

Table 3. Sorbitol, fructose and glucose concentrations in the sciatic nerve of normal and streptozotocin-diabetic rats maintained on a fructose-rich diet for 4 weeks

Animal group	Group code	Glucose ($\mu\text{mol/g}$ wet weight)	Sorbitol ($\mu\text{mol/g}$ wet weight)	Fructose ($\mu\text{mol/g}$ wet weight)
Normal rats				
Untreated ($n=10$)	C	2.65 ± 0.19	0.11 ± 0.04	1.04 ± 0.07
Fructose-fed ($n=12$)	CF	3.25 ± 0.24	0.11 ± 0.02	1.14 ± 0.09
Diabetic rats				
Untreated ($n=7$)	D	3.97 ± 0.84	$0.26 \pm 0.03^{\text{h,c}}$	$2.67 \pm 0.37^{\text{a,c}}$
Fructose-fed ($n=8$)	DF	$6.43 \pm 0.73^{\text{a,c,d}}$	$0.55 \pm 0.08^{\text{a,c,e}}$	$7.61 \pm 1.08^{\text{a,c,d}}$

Results are expressed as mean \pm SEM. ^{a,b} $p < 0.001$, 0.02 versus group C; ^c $p < 0.001$ versus group CF; ^{d,e} $p < 0.005$, 0.02 versus group D rats respectively

Table 4. Motor nerve conduction velocity in normal and streptozotocin-diabetic rats maintained on a fructose-rich diet during treatment with aldose reductase inhibitors and insulin

Animal group	Group code	Motor nerve conduction velocity (m/s) at:		
		0 weeks	2 weeks	4 weeks
Normal rats (fructose-fed)				
Control ($n=6$)	CF	29.5 ± 0.8	32.3 ± 1.7	36.3 ± 1.0
Diabetic rats (fructose-fed)				
Untreated ($n=7$)	DF	$25.6 \pm 0.5^{\text{a}}$	$28.6 \pm 0.5^{\text{c}}$	$28.3 \pm 0.6^{\text{b}}$
Alrestatin-treated ($n=6$)	DFA	26.4 ± 1.3	$31.6 \pm 0.6^{\text{d}}$	$33.1 \pm 0.9^{\text{a,c}}$
ONO-2235-treated ($n=7$)	DFO	$26.9 \pm 0.6^{\text{c}}$	$31.6 \pm 0.7^{\text{d}}$	$33.8 \pm 1.3^{\text{d}}$
Insulin-treated ($n=7$)	DFI	$26.2 \pm 0.5^{\text{a}}$		$33.6 \pm 1.2^{\text{d}}$

Results are expressed as mean \pm SEM. ^{a,b,c} $p < 0.005$, 0.01, 0.05 versus group CF; ^{d,e} $p < 0.005$, 0.01 versus group DF rats respectively

proximately 50%, neither of the inhibitors nor insulin at the doses used completely inhibited aldose reductase at the high blood glucose levels of these rats.

Although the increased accumulation of sorbitol in the sciatic nerve in group DF rats was strongly prevented by the treatment of ONO-2235 and insulin, the effects in the sciatic nerve were observed also in both the retina and erythrocytes of group DF rats (Table 5). Erythrocyte sorbitol levels of all experimental animals were significantly correlated with those of the sciatic nerve ($r=0.86$, $p < 0.001$) and the retina ($r=0.91$, $p < 0.001$).

Discussion

It is now accepted that early metabolic abnormalities in nerves resulting from chronic insulin deficiency and hyperglycaemia, rather than from vascular changes, heavily influence the development of diabetic neuropathy [22]. However, there are numerous proposals for possible pathogenetic metabolic alterations in nerve disorders in diabetes [22, 23].

In the present study, it was revealed that a fructose-rich diet fed to diabetic rats could bring about impaired MNCV which could be prevented by treatment with aldose reductase inhibitors. Although ONO-2235, a new aldose reductase inhibitor, prevented both slowing of MNCV and the elevation of nerve sorbitol concentration, both effects were similar to those of alrestatin and insulin treatment. Although the reduction rate of nerve sorbitol content by treatment with either of the aldose reductase inhibitors or insulin was less in the present study (50–60%) than in other reports (70–90%) [3–7], treatment showed the same improvement of MNCV in our animal models as in these other reports. This low reduction of nerve sorbitol and fructose content by treatment with aldose reductase inhibitors and insulin is explainable by (1) the use of low doses of aldose reductase inhibitors and (2) the direct transport of fructose to the sciatic nerve causing elevated sorbitol and fructose content. In recent experiments with diabetic rats, after 6 months of streptozotocin administration, the animals received ONO-2235 mixed in the food at two different doses (50 and 100 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) for 4 weeks [24]. The MNCV of rats treated with the higher dose was more markedly improved than that of animals treated with the lower dose (42.2 ± 0.8 versus 40.0 ± 0.7 m/s, $p < 0.05$), whilst the MNCV of corresponding untreated diabetic rats was 35.9 ± 1.0 m/s. All these changes appeared to be related to nerve sorbitol content (high dose: 1.45 ± 0.10 ; low dose: 1.63 ± 0.12 ; control: 1.89 ± 0.15 $\mu\text{mol/g}$ wet weight; high dose versus control, $p < 0.01$). Thus, it was expected that the higher doses of aldose reductase inhibitor administered in the present study would cause a more marked effect on the reduction of nerve sorbitol content. In experiments in vivo, most of the administered fructose was converted rapidly into glucose by the liver and approximately 20% of the fructose load was not metabolized [25]. The accumulation of sorbitol and fructose in the retina and lens were observed more markedly in diabetic rats maintained on a fructose-rich diet than in those maintained on a glucose-rich diet [16]. The presumed role of the fructose diet in the present study is on the basis of these data [16; 25]. The increased accumulation of sorbitol and fructose in the diabetic rats maintained on a fruc-

Table 5. Effect of aldose reductase inhibitors and insulin treatment on sorbitol, fructose and glucose concentrations in the sciatic nerve and on sorbitol concentrations in the retina and erythrocytes of streptozotocin-diabetic rats maintained on a fructose-rich diet for 4 weeks

Animal group	Group code	Sciatic nerve ($\mu\text{mol/g}$ wet weight)			Retina	Erythrocytes
		Glucose	Fructose	Sorbitol	Sorbitol ($\mu\text{mol/g}$ wet weight)	Sorbitol (nmol/g haemoglobin)
Normal rats (fructose-fed)						
Control ($n=6$)	CF	2.98 ± 0.18	1.07 ± 0.07	0.11 ± 0.02	0.27 ± 0.10	38.83 ± 7.79
Diabetic rats (fructose-fed)						
Untreated ($n=7$)	DF	5.48 ± 0.69^a	5.96 ± 0.50^a	0.47 ± 0.04^a	1.15 ± 0.01^a	220.00 ± 18.21^a
Alrestatin-treated ($n=6$)	DFA	5.03 ± 0.84^a	$2.70 \pm 0.65^{a,b}$	0.17 ± 0.03^f		
ONO-2235-treated ($n=7$)	DFO	4.89 ± 0.47^b	$3.14 \pm 0.69^{a,b}$	$0.25 \pm 0.05^{a,f}$	0.51 ± 0.11^f	$127.39 \pm 8.97^{a,f}$
Insulin-treated ($n=7$)	DFI	4.36 ± 0.53^c	$2.44 \pm 0.35^{b,f}$	$0.22 \pm 0.03^{a,f}$	0.42 ± 0.15^f	$141.16 \pm 9.00^{a,f}$

Results are expressed as mean \pm SEM.

^{a, b, c, d, e} $p < 0.001, 0.005, 0.01, 0.02, 0.05$ versus group CF; ^{f, g} $p < 0.001, 0.005$ versus group DF rats, respectively

tose-rich diet compared with the untreated diabetic group should accrue from increased polyol pathway activity and hepatic isomerase reaction affecting blood glucose levels. Thus, our findings suggest that the use of aldose reductase inhibitors in animal models has good potential for assessing the role of the polyol pathway in MNCV impairment.

A marked decrease of nerve sorbitol and fructose content in insulin treated diabetic rats was observed despite a small reduction in nerve glucose levels (Table 5). As aldose reductase activity of the polyol pathway is not affected directly by insulin [16, 29], it is likely that the specific activities of several enzymes regulated by insulin are altered and that glucose utilization is diverted to metabolic pathways other than the polyol pathway. In vitro, at physiological glucose concentrations, approximately 3% of the glucose uptake was utilized for sorbitol and fructose synthesis and this increased to as much as 11% at 50 mmol/l glucose concentration [26]. However, extracellular glucose in our study was only approximately 20% of that quoted in the above reference. Nerve sorbitol and fructose synthesis in insulin-treated diabetic rats may be strongly influenced by even a small reduction of nerve glucose content, as was observed in the present study. Moreover, this could explain why neither MNCV nor nerve sugar alcohol content were affected by fructose feeding in normal rats.

Recent histological studies in diabetic neuropathy have demonstrated enlargement of endoneural space as a cause of increased water content [27]. It might be speculated, for example, that if the excess of sorbitol and fructose within the Schwann cell were to leak into the endoneural space, this would contribute to the osmotic force, leading to swelling of the space as well as to shrinkage of the axon and the Schwann cell [22]. As the accumulation of 1 μmol of solute/g wet weight is equivalent to 1 mosmol/l in osmotic pressure [28], osmotic damage could occur with the accumulation of sorbitol and fructose observed in our study. However, the mechanism by which the polyol pathway hyperactivity influences the pathogenesis of diabetic neuropathy is not yet established. Recently, other investigators focussed on

the polyol pathway in conjunction with myo-inositol metabolism in nerves [6, 23, 29]. Thus, peripheral nerve dysfunction in diabetes via polyol pathway hyperactivity may be further complicated by a biochemical mechanism based on the osmotic hypothesis [22, 23, 27].

ONO-2235, has a short circulating half-life (1 h in rats and man) compared with that of sorbinil (4 h in rats) [30], but the same as that of alrestatin [1]. The dose of ONO-2235 used in this study was equal to that used in diabetic patients treated for a 12-week period, when improvement in both nerve conduction velocity and subjective symptoms, such as pain and numbness, correlated well with reduction of erythrocyte sorbitol content [2]. Therefore, the effects of ONO-2235 in the present study could be partially explained on a pharmacological basis.

On the other hand, it is of clinical interest to know whether a good correlation exists between erythrocyte and tissue sorbitol levels in diabetes-associated complications. In the present study, it was confirmed that erythrocyte sorbitol levels were significantly correlated to those of the sciatic nerve ($r=0.86, p<0.001$) and the retina ($r=0.91, p<0.001$) in individual animals. If increased polyol pathway activity is strongly related to the pathogenesis of diabetic complications, our present findings show that erythrocyte sorbitol concentrations may be a useful indicator for the presence of diabetic complications as mentioned by Malone et al. [21].

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